Wasit Journal for Pure Science

Journal Homepage: https://wjps.uowasit.edu.iq/index.php/wjps/index
e-ISSN: 2790-5241 p-ISSN: 2790-5233

The Impact of Different Mediums and The Essential Oil *Eucalyotus Camaldulensis* on The Growth of *Aspergillus* spp

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DOI: https://doi.org/ 10.31185/wjps.465
Received 10 June 2024; Accepted 29 July 2024; Available online 30 September 2024

ABSTRACT: *Eucalyptus* Essential oils has beneficial properties as fungicidal, bactericidal, insecticidal and other therapeutic properties. The result also showed that the best medium for growth of isolated fungi was PDA followed by SDA, because they provide a number of nutrient that are necessary for the growth of fungi, Potatoes are a great ingredient to media like the widely used Potato Dextros Agar (PDA). These results show that the effects of the essential oils on the tested organisms increase with concentration, with the lowest radial growth recorded at 100 percent on account of *Aspergillus niger* (29.50 mm) and the highest at 25% on account of *Aspergillus fumigatus* (68.17 mm). When compared to the control, all treatments had a considerable impact on the tested fungi growth. The results also showed that *Aspergillus fumigatus* had a mean radial growth of (57.683 mm), whereas *Aspergillus nige*, with a mean radial growth of (52.583 mm), was the more sensitive fungus. *Aspergillus flavus* came in second with a mean radial growth of (55.042 mm). *Aspergillus fumigatus* had the lowest mean of inhibition percentage (43.90%) and *Aspergillus niger* had the greatest mean (47.83%).

Keywords: *culture media, Aspergillus, Eucalyptus, essential oil.*

1. INTRODUCTION

Aspergillus species are opportunistic fungus that become harmful when their host's immunity is debilitated Among the many species of *Aspergillus*, the four most common are *Aspergillus fumigatus* followed by *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus clavatus*. Then again, non-*fumigatus Aspergillus*-related contagious infections have become more prevalence. Human infections with *Aspergillus* species can result in a large number of ailments, from direct angioinvasion to hypersensitive reactions, contingent upon the host's safe system [1].

Around 250 species and 40 subspecies of the saprophytic fungus *Aspergillus* spp. can infections humans. This ecological factor is the cause of common infections, especially opportunistic respiratory tract infections in immunocompromised individuals. Infections can also be caused by *Aspergillus circumdati*, *Aspergillus candidi*, *Aspergillus terrei*, *Aspergillus nidulantes*, *Aspergillus warcupi*, and *Aspergillus ornati* subspecies, however *Aspergillus fumigatus* is the most common causative agent. *Aspergillus* species can create saprophytic, allergic, and invasive aspergillosis, which are the clinical symptoms of the disease [2],[3].

In any case, those with debilitated resistant systems and insufficient immune response of *Aspergillus* resistance are more helpless against invasive aspergillosis (IA), a lethal illness with a high morbidity and mortality rate. The passing rate from invasive aspergillosis is still somewhere in the range of 30 and 70 percent because of difficulties with diagnosis, a small selection of antifungal medications, and a lack of effective antifungal vaccinations [4],[5],[6].

Rich sources of bioactive chemicals viewed in plants have been showed as useful against various illnesses and diseases [1]. Leaves of *Eucalyptus camaldulensis* are known to possess several biological and pharmacological activities, including antifungal activity against several pathogenic contagious fungi [7],[8].

When four normal form mold fungi (*Aspergillus flavus*, *Aspergillus nige*, *Aspergillus terreus*, and *Fusarium culmorum*) were developed, the essential oil from *Eucalyptus camaldulensis* were tested for its antifungal activity. The results showed that the oil had great antifungal activity, with *Aspergillus niger* showing the highest percent of inhibition (91.66%) [9]. Therefore, this study was to assess the impact of *Eucalyptus camaldulensis* essential oil and different mediums against growth *Aspergillus* spp. that cause aspergillosis.

2. MATERAL AMD METHODS

2.1 Source of culture media

The culture media (Potato Dextros Agar, Sabouraud Dextrose Agar, Malt Extract Agar and Yest Extract Agar) utilized in this study were purchased from a medical equipment in the province of Wasit.

2.2 Source of essential oil

The Ministry of Science and Technology, Directorate of Agrarian Research supplied the essential oil.

2.3 Effect of different media on the growth of *Aspergillus* spp.

In this work, four culture media — potato dextrose agar (PDA), sabouraud dextrose agar (SDA), malt extract agar (MEA), and yeast extract agar (YEA) were utilized. They were prepared as per the manufacturer's instructions (39gm, 62gm, 20gm and 23gm) respectively. They were totally dissolved in 1 liter distilled water and then, add 0.5 ml/L of Cycloheximide and o.o5 g/L of chloramphenical to prevent growth microorganism, than put in autoclaved at 121 degrees Celsius for fifteen minutes at a pressure of less than fifteen pounds. Twenty milliliters of each of these media were placed in sterile Petri plates measuring nine centimeters. For approximately sixty minutes, the media were left to harden at room temperature. In this investigation, a disk (5 mm) containing a developed pure culture of *Aspergillus* spp. was inoculated independently in each of the investigated conditions and incubated at 25°C. The province diameters of the *Aspergillus* species utilized in this study in all media were measured after 7 days after immunization. There were three replications of each treatment in a completely randomized setup [10].

2.4 Effect of essential oil on radial growth of Aspergillus spp. by poisoned food technique

To assess the effect of essential oil on Aspergillus spp. development using the poisoned food technique, 90 ml of autoclaved PDA medium was combined with 10 ml of each concentration and then put into 9cm petri dishes. As a control, Petri plates filled with medium free of oil were utilized. A 10 mm diameter mycelial disc of the tested fungus was used to inoculate each dish, and it was then incubated at 25 °C for the predetermined measure of time. The province's diameter was measured, and the findings were noted. The accompanying formula was used to get the inhibition percentage: Control growth - test growth/control growth x 100 equals the inhibition percentage [11].

3. RESUITS AND DISCUSSION

3.1 Effect of different media in growth of Aspergillus spp.

Following a 7-day brooding period, the effects of different media (PDA, SDA, MEA, and YEA) on the development rate of *Aspergillus* species were displayed in Table (1) and Figure (1). The tested contagious colonies on PDA, SDA, MEA, and YEA had settlement diameters of (78.33, 76.20, 48.83, and 38.33 mm) for *Aspergillus fumigatus*, (79.50, 77.67, 44.00, and 34.50 mm) for *Aspergillus niger*, and (79.13, 74.77, 42.83, and 28.33 mm) for *Aspergillus flavu*s.

As indicated by the information, PDA and SDA were the two most effective development media for the growths under examination. Moreover, the results demonstrated that the PDA culture medium far outperforms any remaining examined media in terms of advancing the spiral growth of the organisms under test. When it came to outspread growth, PDA and the fungus A. niger had the most growth, measuring 79.50 mm, while YEA and the fungus *Aspergillus flavus* had the lowest growth, measuring 28.33 mm. Significant differences were tracked down in this investigation (p value = 0.001).

Table 1. Radial growth of *Aspergillus* spp, according to the type of media (PDA, SDA, MEA, YEA)

Type of Fungi	Colony diameter in (mm) Type of media (M)				Mean Fungi	
(F)	PDA	SDA	MEA	YEA		
Aspergillus niger	79.50	77.67	44.00	34.50	58.92 b	
Aspergillus fumigatus	78.33	76.20	48.83	38.33	60.43 a	
Aspergillus flavus	79.13	74.77	42.83	28.33	56.27 c	
LSD $_{F*M}$	0.73				LSD $_{F}$	0.36
Mean media	78.99 a	76.21 b	45.22 c	33.72 d		
LSD $_{M}$	0.42					

Each value was a mean of three replicates. Significant LSD (0.05)

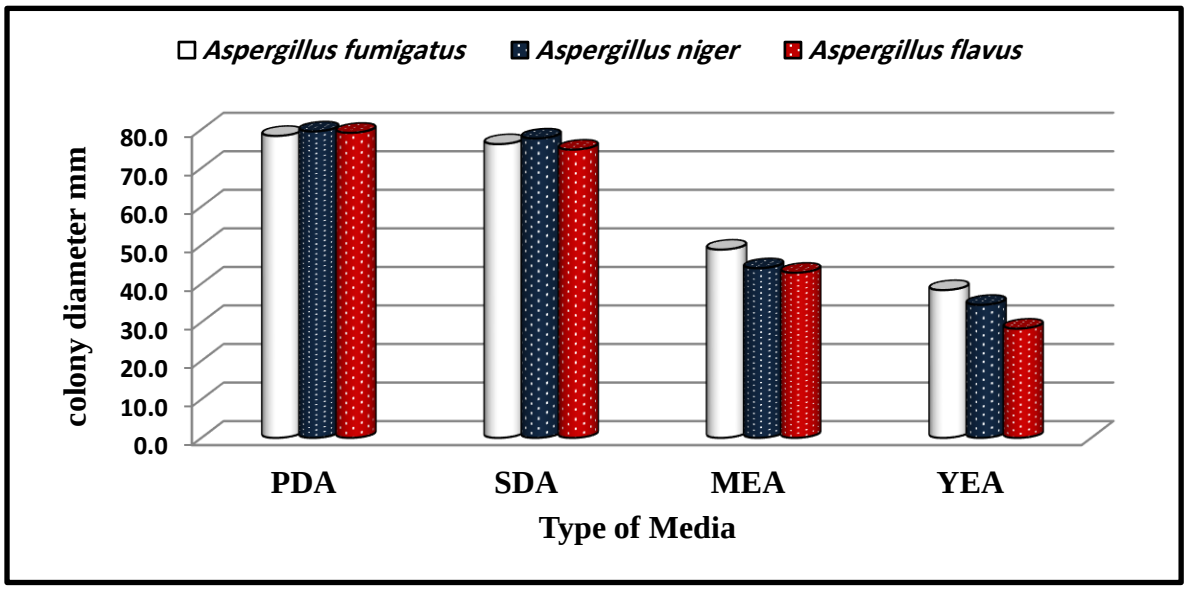

FIGURE 1. Radial growth of *Aspergillus* niger, according to the type of media **(**a- PDA b- SDA c- MEA d- YEA)

The findings of this study showed that the growth medium fundamentally affected the development of the three fungal species. The medium YEA showed the lowest growth rate, while PDA showed the highest growth rate. These differences in the nourishing components of the tested media might have contributed to the observed growth differences in the targeted fungi.

PDA's straightforward composition and ability to facilitate the mycelial growth of a wide variety of growths make it one of the most often used culture medium. PDA is the greatest medium for mycelial growth, as indicated by a number of experts [12], [13].

PDA and SDA were said to be the ideal media for mycelia development by a number of workers [14]. The findings of this investigation were agreement with those of [15] who examined the effect of various media on *Aspergillus terreus* development and concluded that PDA was the most effective medium, followed by SDA and MEA.

After seven days of incubation at 25±1°C, another study was carried on a mission to assess the influence of Potato Dextrose Agar (PDA), Czapek's Dox, Yeast Extract Agar (YEA), and Lignocellulose Agar (LCA). The results demonstrated that the tested organisms exhibited most extreme settlement development on PDA [16].

As per certain reports [17], fungal isolates cultivated on PDA media have demonstrated superior spore creation and have enhanced their capacity, which is consistent with the findings of our investigation.

Aspergillus niger cultivated on PDA media outgrew CYA (Czapek's Dox + Yeast Extract Agar), as per [18].

[19], study examined the effects of three different culture media on *Aspergillus* species practicality and spore creation. It found that PDA produced the most development and spore creation, followed by SDA, and that result was consistent with our findings.

Extra research examined the effect of several nourishing variables on the development of *Aspergillus*. [20],[21],[22].

3.2 Effect of essential oil on growth of *Aspergillus* spp. by poisoned food technique

The findings of the experiment using poisoned food to examine the effects of essential oils on the development of *Aspergillus* species are shown in Tables (2, 3), and Figure (2). These results show that the effects of the essential oils on the tested organisms increase with concentration, with the lowest radial growth recorded at 100 percent on account of *Aspergillus niger* (29.50 mm) and the highest at 25% on account of *Aspergillus fumigatus* (68.17 mm).

When compared to the control, all treatments had a considerable impact on the tested fungi growth. The results also showed that *Aspergillus fumigatus* had a mean radial growth of (57.683 mm), whereas *Aspergillus niger* with a mean radial growth of (52.583 mm), was the more sensitive fungus. *Aspergillus flavus* came in second with a mean radial growth of (55.042 mm). *Aspergillus fumigatus* had the lowest mean of inhibition percentage (43.90%) and *Aspergillus niger* had the greatest mean (47.83%).

Table 2. Effect of essential oil of Eucalyptus on *Aspergillus* spp. growth by poisoned food technique

Treatment		**Radial growth (mm)**			
Extract	**Concentrations (%)**	***A.niger***	***A. flavus***	***A. fumigatus***	**Means**
Essential oil	100	29.50	30.33	35.33	31.722
	50	38.50	40.33	41.23	40.022
	25	60.33	65.50	68.17	64.667
	Control	82.00	84.00	86.00	84.00
LSD (0.05)		0.7264			0.4194
Means		52.583	55.042	57.683	
LSD (0.05)		0.3632			

Each value is a mean of three replicates

Table 3. Percentages of inhibition of *Aspergillus* spp. growth by poisoned food technique

Treatment		**Inhibition %**			
Extract	**Concentrations (%)**	***A.niger***	***A. flavus***	***A. fumigatus***	**Means**
Essential oil	100	64.02	63.89	58.91	62.28
	50	53.05	52.98	52.05	52.69
	25	26.42	22.02	20.74	23.06
LSD (0.05)		1.021			0.589
Means		47.83	46.29	43.90	
LSD (0.05)		0.589			

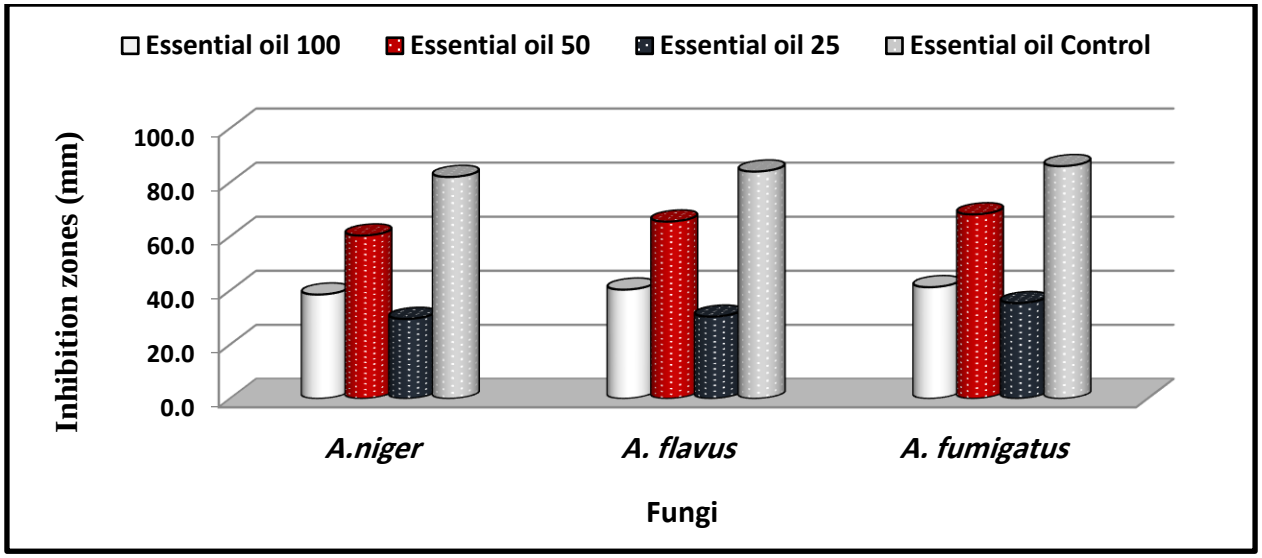

FIGURE 2. Effect of essential oil on *A.fumigatus* by poisoned food technique a- 100 , b- 50 , c- 25% , d- control

The study's findings indicated that the effectiveness of essential oil varies depending on the fungus species and concentration. These findings aligned with those of [23] who discovered that the essential oil of *Eucalyptus urophylla* against *Aspergillus niger* increased in effect as the oil concentration increased. They also demonstrated that the mechanisms underlying the reduction of mycelium growth were likely caused by the main compound, 1,8-cineole, which accounted for 66.31% of the total.

The antibacterial activity of *Eucalyptus* essential oil was investigated by [25] who demonstrated that the oil's activity stems from the presence of 1,8-cineole, which is the oil's active constituent and causes a variety of pharmacological effects. Numerous studies also revealed that 1,8-cineole, the primary component of *Eucalyptus* oil, had antifungal properties [26],[27].
The antifungal activity of eleven commercial Eos-clove, *Eucalyptus*, geranium, half breed lavender, lavender, lemon, lemongrass, neroli, oregano, tea tree, and red thyme — was studied by [28] against clinical isolates of *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger*. The study was conducted in comparison with AMB and ITZ. The findings encourage the development of novel EO-based complementary or alternative medicines by demonstrating the antibacterial potential of some EOs.

4. CONCLUSION

In this study, we investigate the antifungal activity of essential oil of *Eucalyptus camendulensis* plant and different mediums (PDA, SDA, MEA and YEA) against aspergillus spp. that isolated from patients. We found that the essential oils of *E. camendulensis* plant showed an interesting biological activity on *Aspergillus* spp. As indicated by the information, PDA and SDA were the two most effective development media for the growths under examination. Moreover, the results demonstrated that the PDA culture medium far outperforms any remaining examined media in terms of advancing the spiral growth of the organisms under test**.**

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