Antifungal Activity of the Monoterpenes Carvacrol, *p*-Cymene, Eugenol, and Iso-Eugenol When Applied to Wood against *Aspergillus flavus*, *Aspergillus niger,* and *Fusarium culmorum*

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This work was designed to evaluate the bioactivity of four monoterpenes, namely carvacrol, *p*-cymene, eugenol, and iso-eugenol, applied to wood blocks from *Pinus sylvestris* sapwood using the vapor method against *Aspergillus flavus*, *Aspergillus niger*, and *Fusarium culmorum*. These monoterpenes were prepared at 20, 40, 60, 80, and 100 µL/mL. The highest fungal inhibition percentage (FIP, 24.4%) against the growth of *A. flavus* was observed for p-cymene when applied to a wood sample at 100 µL/mL. The highest FIPs observed against the growth of *A. niger* were 21.5% and 16.3%, by p-cymene and iso-eugenol, respectively, at 100 µL/mL. The highest FIPs observed against the growth of *F. culmorum* were 41.5 and 27.0% by the application of carvacrol at 100 µL/mL and 80 µL/mL, respectively. This study showed the importance of monoterpenes for antifungal activity and may contribute to the most rational use of these compounds as antimicrobial agents for wood protection.

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INTRODUCTION

Wood is a naturally occurring, renewable, extremely adaptable, and high-performing material that has been widely utilized by humans from their beginning. Additionally, it holds the greatest amount of carbon trapped in terrestrial ecosystems. However, because of its structure and chemical components, wood is vulnerable to biodeterioration, with fungi being the primary degraders (Goodell *et al.* 2008; Brischke and Alfredsen 2020; Afifi *et al.* 2023). Mold stains can cause damage to wood and other organic materials; their activity results in the discoloring of wood, which detracts from its aesthetic value even if they are not seriously damaging structural integrity (Allsopp *et al.* 2004; Kim *et al.* 2020; Eldeeb *et al.* 2022; Taha *et al.* 2021; Mansour *et al.* 2023). Certain environmental factors, such as moisture content above 20%, oxygen availability, and temperature between 15 and 45 °C, make wood vulnerable to fungal infestation. The primary target of fungal decay is outdoor wooden structures. The infestation can greatly

reduce the mechanical and aesthetic qualities of the wood and shorten its service life (Zabel and Morrell 2012; Meyer and Brischke 2015).

Secondary metabolites of plants called monoterpenes are commonly utilized in industrial processes as starting points for significant fragrance compounds including (−)-menthol and vanillin. Nevertheless, monoterpenes' physicochemical characteristics make it challenging to convert them conventionally into scents with additional value (Soares-Castro *et al.* 2020). Because of their low boiling temperatures, monoterpenes are the primary components of most plant essential oils and are responsible for the distinctive odorous qualities of plants. For example, geranyl pyrophosphate, the common acyclic C10 intermediate of the isoprenoid route, is the starting point for their biosynthesis (Rehman *et al.* 2016; Gershenzon and Croteau 2018). Monoterpene hydrocarbons and oxygenated monoterpenes are the two main categories of monoterpenes. Alcohols, aldehydes, ketones, ethers, and acids are included in the latter category (Zuzarte and Salgueiro 2015; Soares-Castro *et al.* 2020; Yingngam 2022). Certain monoterpenes have inherent pesticidal qualities that make them suitable starting compounds for the development of safe, efficient, and completely biodegradable insecticides as well as possible substitutes for pesticides (Khursheed *et al.* 2022; Gupta *et al.* 2023). Numerous fungicidal actions of monoterpenes (Wuryatmo *et al.* 2003; Cárdenas-Ortega *et al.* 2005), and other characteristics, are possessed.

In common practice, the durability issue with wood has been addressed by preservative treatments such as creosote, pentachlorophenol, and inorganic arsenic (Cheng *et al.* 2008). However, when these preservatives are utilized over time, they cause serious issues with pollution to the environment and human health. Thus, it becomes increasingly important to look for bioactive chemical compounds from plants that are natural, safe, and do not pollute as a substitute for synthetic preservatives (Loh *et al.* 2011; Hu *et al.* 2015).

During the authors' continuous search for potential antifungal substances, four monoterpenes, namely carvacrol, *p*-cymene, eugenol, and iso-eugenol were applied to wood samples and evaluated for their antifungal activity against three plant pathogenic fungi *Aspergillus flavus*, *Aspergillus niger*, and *Fusarium culmorum*. Monoterpenes, including geraniol, myrcene and thymol were observed to have promising antifungal activity against four plant pathogenic fungi *Asperigallus niger*, *Rhizoctonia solani*, *Fusarium oxysporum*, and *Penecillium digitatum* (Marei *et al.* 2012). Among 20 compounds, the antifungal tests revealed that cuminaldehyde, β-citronellol, nerol, geraniol, citral, and *α*-terpineol exhibited strong antifungal effects against *Botryosphaeria dothidea* (Zhang *et al.* 2018). Carvacrol was found to be the most potent of the 41 pure monoterpenes against the wood white-rot fungi *Trametes hirsuta*, *Schizphylhls commune*, and *Pycnoporus sanguineus*. This means that carvacrol may be used as a natural fungicidal agent in the treatment of wood preservation (Zhang *et al.* 2016). Strong antifungal activity was demonstrated by cinnamaldehyde, *α*-methyl cinnamaldehyde, (*E*)-2-methylcinnamic acid, eugenol, and isoeugenol against the brown-rot fungus *Laetiporus sulphureus* and the white-rot fungus *Lenzites betulina* (Cheng *et al.* 2008). Additionally, it was discovered that natural compounds have fungicidal properties against the fungi that cause wood decay. Of these natural compounds, the most potent antifungal ones were eugenol, *α*-cadinol, *τ*-muurolol, *τ*-cadinol, *γ*-cadinene, cryptomeridiol, chamaecynone, cinnamaldehyde, and ferruginol (Wang *et al.* 2005 a,b; Cheng *et al.* 2006; Yen and Chang 2008).

Thus, this study aimed to find a botanical-based compound with potential antifungal activity against wood-decay pathogenic fungus. To reach this goal, the study investigated the fungicidal activity of four monoterpenes, carvacrol, *p*-cymene, eugenol, and iso-eugenol when applied to wood samples against three pathogenic fungi and compared the antifungal activity potential of these four monoterpenes against each pathogenic type of fungi.

EXPERIMENTAL

Materials

Monoterpenes

Four monoterpenes (Fig. 1), carvacrol, *p*-cymene, eugenol, and iso-eugenol, were obtained from Sigma-Aldrich (Merck). The monoterpenes were prepared at concentrations of 20, 40, 60, 80, and 100 µL/mL. The respective amount of monoterpene was diluted in 10% dimethyl sulfoxide (10% DMSO and 90% sterile distilled water), and 0.5 mL of Tween 80 (polysorbate-80) as emulsifier was added (Salem *et al.* 2016; Mohareb *et al.* 2023).

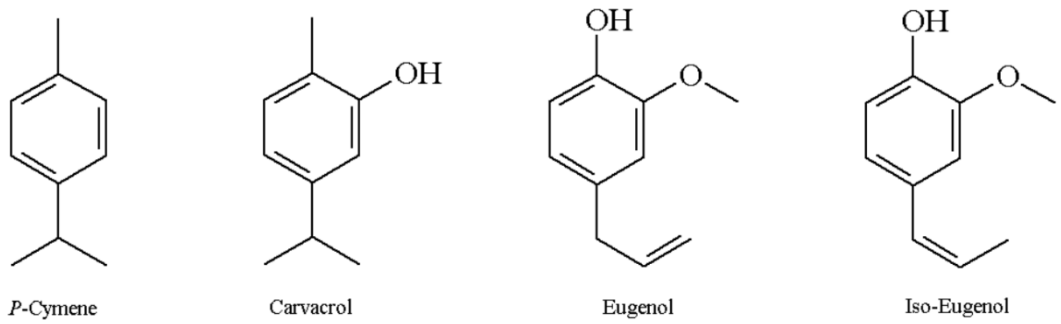


Fig. 1. Chemical structures of the monoterpenes

Fungi

The antifungal bioassays of the four monoterpenes, carvacrol, *p*-cymene, eugenol and iso-eugenol were conducted using three molds (*Aspergillus flavus* AFl375, *Aspergillus niger* Ani245, and *Fusarium culmorum* Fcu761) and accession numbers in Gen Bank, MH355958, MH355955, and MH355957, respectively (Abo Elgat *et al.* 2020; Elshaer *et al.* 2024).

Methods

Vapor treatment of wood with the monoterpenes for fungi inhibition

Pinus sylvestris wood, which is widely used in Egyptian woodworking, was selected for the work as an expensive imported wood. Therefore, the staining mold fungi can grow over the wood when the appropriate conditions—namely, relative humidity, moisture content, and temperature—are met. Wood blocks from *P. sylvestris* sapwood (Mohareb *et al.* 2023) in the dimension of 0.5 × 2 × 2 cm were vapor-treated with each of the prepared monoterpenes at the previous concentrations using the evaporation method

(López *et al.* 2005; Nedorostova *et al.* 2009). Wood samples were put in Petri dishes that contained 8 layers of Wattman No. 1 filter paper overlaid by a mesh (polyethylene spacer). The dishes were autoclaved at 121 °C for 20 min and left to cool, then each monoterpene compound with the respective concentration was impregnated over the filter papers (three Petri dishes for every monoterpene and concentration) and kept for 48 h to allow the monoterpene evaporation, which was subsequently absorbed by the wood samples.

In vitro antifungal activity of treated wood with monoterpenes

The antifungal activity of wood treated with four monoterpenes samples against the growth of *A. flavus*, *A. niger*, and *F. culmorum* was achieved (Taha *et al.* 2019; Elshaer *et al.* 2024). A 15-day-old PDA culture of each fungus was prepared. Three wood samples were used for each concentration. Following the application of each monoterpene compound to wood samples, a Petri dish containing PDA culture was inoculated with a disc (5-mm diameter) of each fungus, and the samples were then incubated for a week at 25 ± 1 °C. As an alternative, 10% DMSO and SDW (1:1 v/v) were combined in the control sample, while fluconazole (0.31%) was used as a positive control. The inhibition zones (IZs, mm) of the monoterpenes around the treated woods against each fungus were measured and recorded (Ali *et al.* 2021).

The mycelial growth inhibition percentage was measured with the following formula (Correa-Pacheco *et al.* 2017; Shakam *et al.* 2022): MGI = $[(A_c - A_t) / A_c] \times 100$; where MGI is mycelial growth inhibition and A_c and A_t are average diameters of the fungal colony of the control and treatment, respectively.

After two weeks of inoculation, the visual observation of the fungal growth extent was visually evaluated by the naked eye in accordance with the GOST 9.048–75 (1975) standard, which ranged from 0 (mycelium growth more intense than control) to 5 (no growth).

Statistical analysis

The analysis of variance (ANOVA) tool in SAS version 8.2 was used to statistically examine data from the application of monoterpenes on wood samples against the growth of each fungus. Duncan's Multiple Range Test at Alpha 0.05 was used to measure the differences among the means. The IC_{50} (half-maximal inhibitory concentration) value is a measure of the concentration of a compound required to inhibit each fungal growth by 50%. These IC_{50} values were determined using Probit analysis (Finney 1952).

RESULTS AND DISCUSSION

Visual Observation, Inhibition Zones, and the Fungal Growth on Wood-Treated Monoterpenes

Fungicidal activity was estimated by fungal growth retardation using the visual observation-determined marks (Table 1) and the antifungal bioassay (Figs. 2, 3, and 4). The highest number (4) in Table 1 shows a very marked retardation (colony < 25% of controls) of fungi, especially carvacrol at 100 µL/mL with *F. culmorum*, *p*-cymene at 100 µL/mL with *A. flavus* and *F. culmorum*, and eugenol and iso-eugenol at 100 µL/mL with *F. culmorum*.

Table 1. Marks of Fungal Growth Retardation

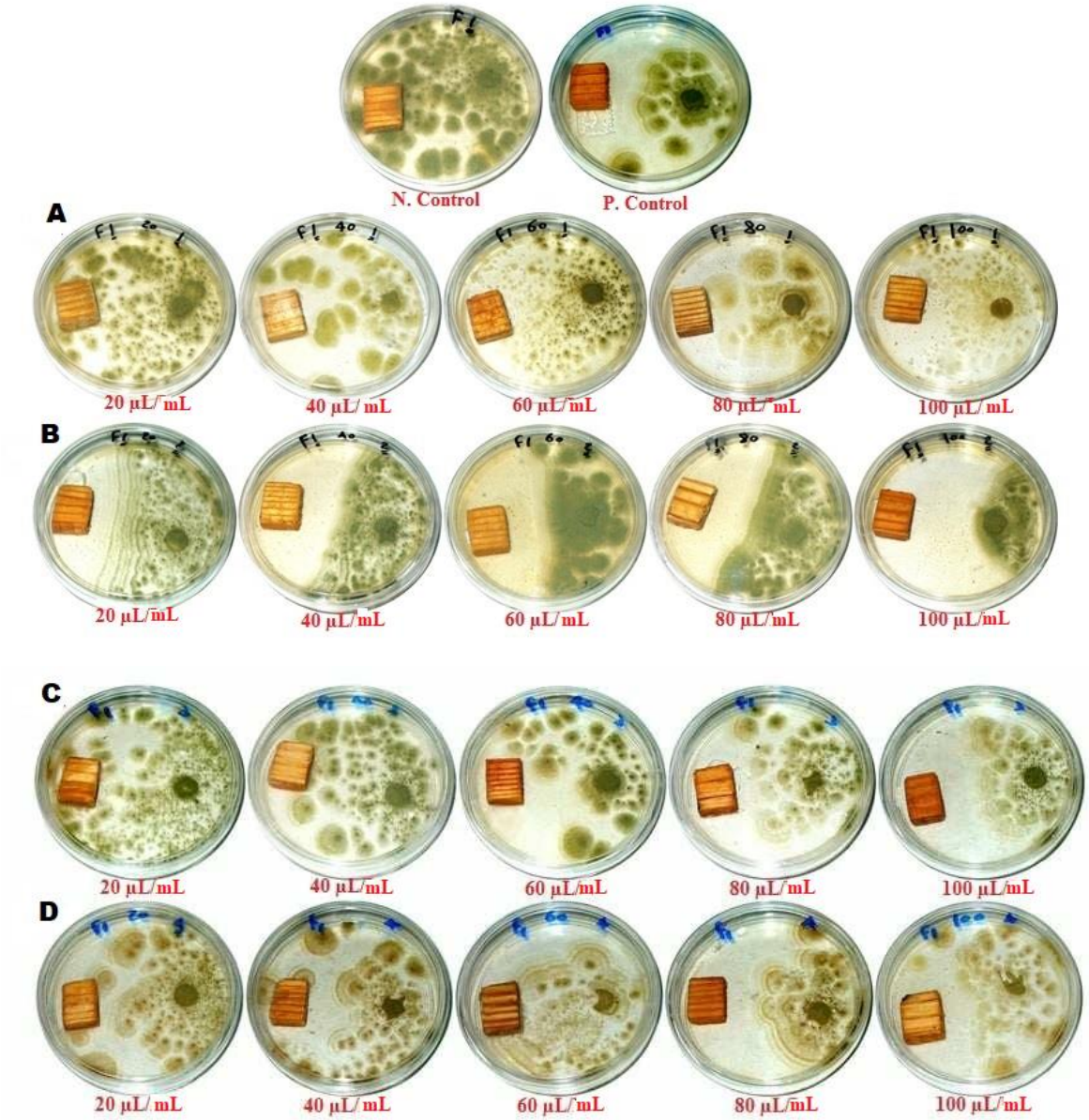
Treatments	Concentration	Fungal Growth Retardation Marks*		
		A. flavus	*A. niger*	*F. culmorum*
Carvacrol	20 µL/mL	1	0	2
	40 µL/mL	2	1	2
	60 µL/mL	2	2	2
	80 µL/mL	2	2	3
	100 µL/mL	3	3	4
p-Cymene	20 µL/mL	2	1	1
	40 µL/mL	2	1	2
	60 µL/mL	2	2	2
	80 µL/mL	2	2	2
	100 µL/mL	4	3	4
Eugenol	20 µL/mL	1	1	1
	40 µL/mL	1	1	1
	60 µL/mL	2	2	2
	80 µL/mL	2	2	2
	100 µL/mL	3	3	4
Iso-eugenol	20 µL/mL	1	1	2
	40 µL/mL	2	2	2
	60 µL/mL	2	2	2
	80 µL/mL	3	2	3
	100 µL/mL	3	3	4

* Values are measured according to: (GOST-9.048-89 1975; Humar and Pohleven 2005; Krivushina *et al.* 2022). 0: growth more intense than control, 1: normal growth, insignificant retardation (area of colony ≥ 90% of area of controls); 2: visible signs of retardation (colony < 90% and ≥ 60% of controls); 3: pronounced retardation (colony < 60% and ≥ 25% of controls); 4: very marked retardation (colony < 25% of controls); 5: no growth

As shown in Fig. 2 and Table 2, there was enormous or massive growth of *Aspergillus flavus* on an untreated wood sample, but this growth decreased when the wood samples were treated with the standard fungicide (fluconazole, 0.31%). The highest inhibition zone (IZ) values were 22.00, 19.00, and 17.00 cm by *p*-cymene, eugenol, and iso-eugenol at 100 µL/mL, respectively, as well as *p*-cymene at 80 µL/mL with an IZ value of 17.3 mm, compared to fluconazole (17.7 mm).

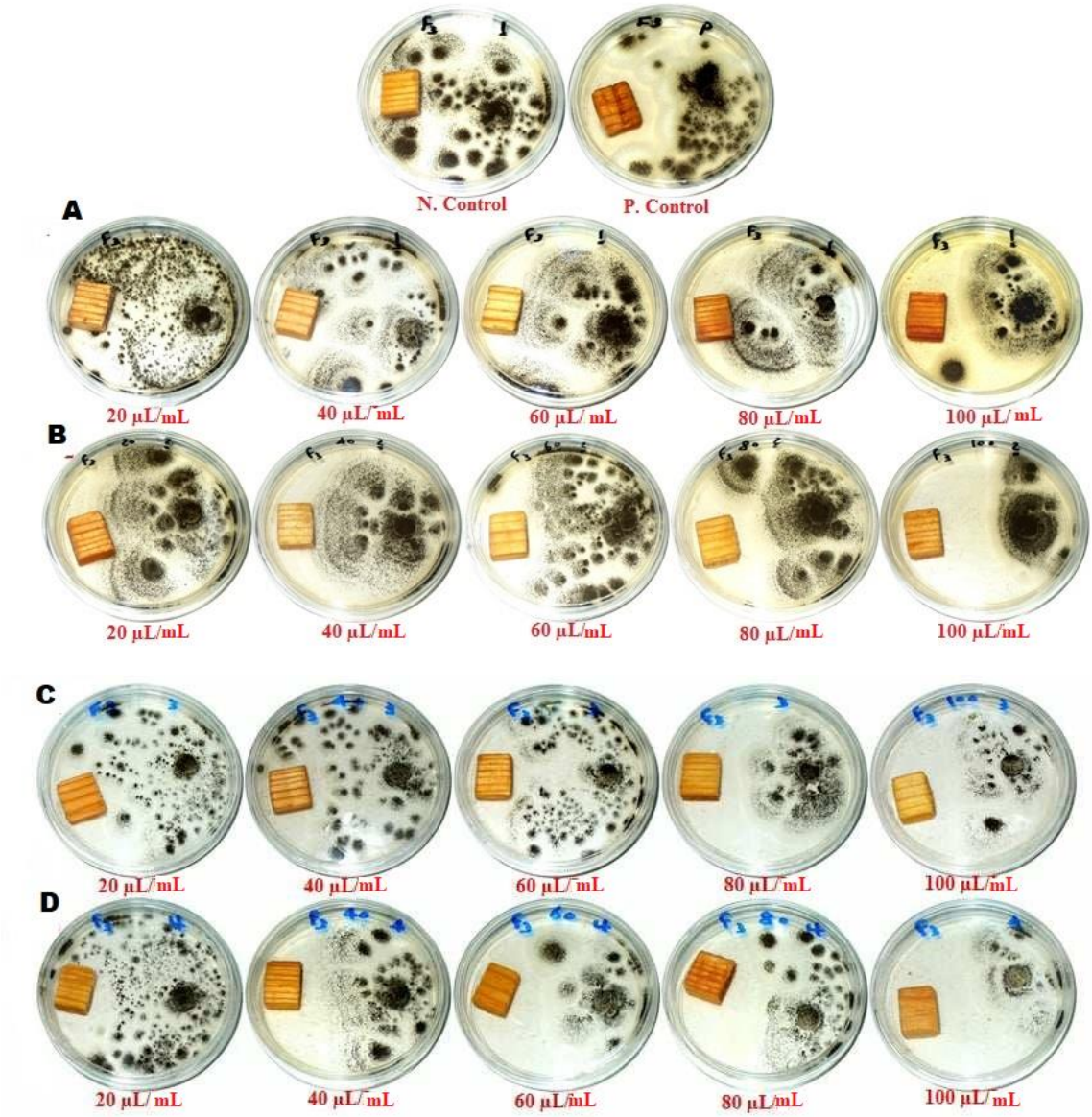
Figure 3 and Table 2 present the antifungal activity of monoterpene-treated wood against the growth of *A. niger*. The highest IZ values were 19.3 and 14.7 mm as wood samples treated with *p*-cymene and iso-eugenol, respectively, at 100 µL/mL. Additionally, at 80 µL/mL, the treated wood samples showed IZ values of 10.3 and 10.7 mm by *p*-cymene and iso-eugenol, respectively, compared to fluconazole (7.3 mm).

Figure 4 and Table 2 present the antifungal activity of monoterpene-treated wood against the growth of *Fusarium culmorum*. The highest IZs were recorded by the application of carvacrol at 100 and 80 µL/mL with values of 37.3 and 24.3 mm, respectively. These were followed by eugenol, *p*-cymene, and iso-eugenol with values of 21.3, 20.7, and 20.3 mm, respectively, and iso-eugenol at 80 µL/mL with IZ 19.3 mm, compared to fluconazole (17.3 mm).



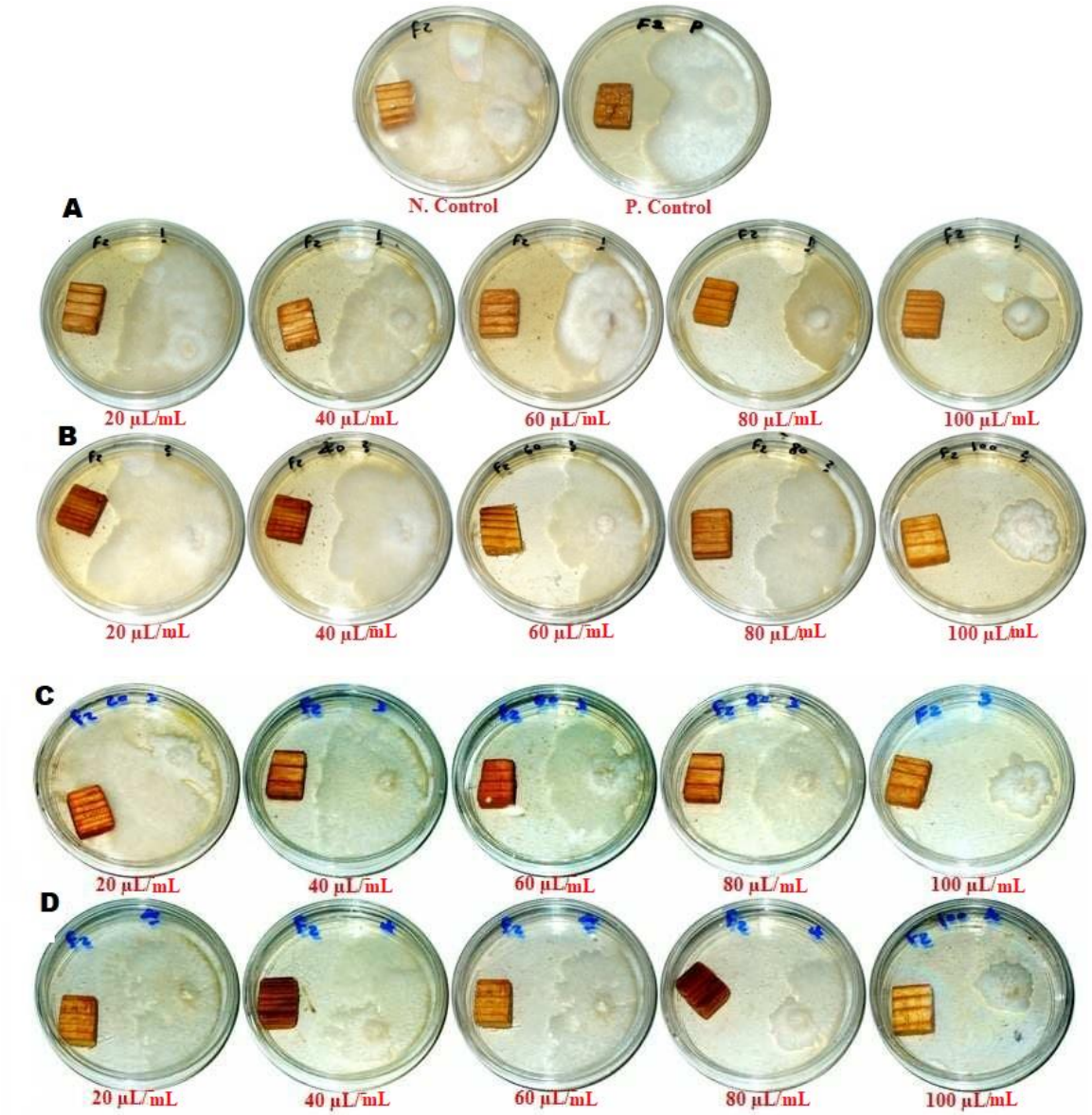
A: Carvacrol; B: *p*-cymene; C: Eugenol; D: Iso-eugenol

Fig. 2. Antifungal activity of monoterpenes-treated wood against the growth of *Aspergillus flavus*



A: Carvacrol; B: *p*-cymene; C: Eugenol; D: Iso-eugenol

Fig. 3. Antifungal activity of monoterpenes-treated wood against the growth of *Aspergillus niger*



A: Carvacrol; B: p-cymene; C: Eugenol; D: Iso-eugenol

Fig. 4. Antifungal activity of monoterpenes-treated wood against the growth of *Fusarium culmorum*

Table 2. Fungal Inhibition Zones and Growth After 14 Days Following the Application of Carvacrol, *p*-cymene, Eugenol, and Iso-eugenol on Wood Samples

Compound	Con-centration	*Aspergillus flavus*		*Aspergillus niger*		*Fusarium culmorum*	
		IZ-14 days (mm)	Growth-14 days	IZ-14 days	Growth-14 days	IZ-14 days	Growth-14 days
DMSO	10%	0.00	12.33 ± 2.51a	0.00i	10.66 ± 1.15a	0.00o	12.33 ± 3.21a
Fluconazole	0.31%	17.66 ± 2.08bc	0.00d	7.33 ± 1.15d	0.00e	17.33 ± 2.51f	0.00b
Carvacrol	20 µL/mL	0.00g	10.00 ± 1.00b	0.00i	4.33 ± 0.57b	9.33 ± 0.57kl	0.00b
	40 µL/mL	2.33 ± 0.57g	0.00d	0.00i	2.33 ± 0.57c	11.66 ± 1.52ij	0.00b
	60 µL/mL	4.66 ± 0.57f	0.00d	0.00i	1.33 ± 0.57d	18.66 ± 1.15ef	0.00b
	80 µL/mL	6.33 ± 0.57f	0.00d	0.00i	0.66 ± 0.57e	24.33 ± 0.57b	0.00b
	100 µL/mL	9.66 ± 0.57e	0.00d	5.66 ± 0.57ef	0.00e	37.33 ± 2.08a	0.00b
p-Cymene	20 µL/mL	10.33 ± 0.57e	0.00d	0.00i	2.66 ± 0.57c	7.66 ± 0.57l	0.00b
	40 µL/mL	12.66 ± 1.15d	0.00d	0.00i	1.66 ± 0.57d	10.33 ± 0.57jk	0.00b
	60 µL/mL	16.33 ± 0.57c	0.00d	5.33 ± 0.57ef	0.00e	12.66 ± 1.15hi	0.00b
	80 µL/mL	17.33 ± 1.15bc	0.00d	10.33 ± 0.57c	0.00e	15.33 ± 0.57g	0.00b
	100 µL/mL	22.00 ± 2.00a	0.00d	19.33 ± 1.15a	0.00e	20.66 ± 1.15cd	0.00b
Eugenol	20 µL/mL	0.00g	1.66 ± 0.57c	0.66 ± 0.57i	0.00e	0.00o	1.33 ± 0.57b
	40 µL/mL	0.00g	1.00 ± 1.00cd	2.33 ± 0.57h	0.00e	4.33 ± 0.57m	0.00b
	60 µL/mL	5.50 ± 0.707f	0.00d	4.50 ± 0.707fg	0.00e	9.50 ± 0.707kl	0.00b
	80 µL/mL	8.75 ± 3.94e	0.00d	6.00 ± 1.41e	0.00e	11.75 ± 1.25ij	0.00b
	100 µL/mL	19.00 ± 1.00b	0.00d	10.33 ± 0.57c	0.00e	21.33 ± 1.15c	0.00b
Iso-eugenol	20 µL/mL	0.00g	1.00 ± 0.00cd	1.33 ± 0.57hi	0.00e	2.33 ± 0.57n	0.00b
	40 µL/mL	0.00g	0.66 ± 0.57cd	3.66 ± 0.57g	0.00e	10.66 ± 0.57jk	0.00b
	60 µL/mL	0.00g	0.00d	6.33 ± 0.57de	0.00e	14.33 ± 0.57gh	0.00b
	80 µL/mL	15.33 ± 0.57c	0.00d	10.66 ± 1.15c	0.00e	19.33 ± 0.57de	0.00b
	100 µL/mL	17.00 ± 1.00bc	0.00d	14.66 ± 0.57b	0.00e	20.33 ± 0.577cde	0.00b

Means with the same letter are not significantly different according to Duncan's Multiple Range Test at 0.05 level of probability.

The Fungal Inhibition Percentages and the IC_{50}

The fungal inhibition percentage (FIP%) is shown in Table 3. Compared to fluconazole (FIP 19.6%), *p*-cymene, when applied to a wood sample at 100 µL/mL, achieved the highest FIP (24.4%) against the growth of *Aspergillus flavus*, followed by eugenol at 100 µL/mL (21.1%) and *p*-cymene at 80 µL/mL (19.2%). Additionally, iso-eugenol at 100 µL/mL and 80 µL/mL resulted in FIP values of 18.9% and 17.0%, respectively, and *p*-cymene at 60 µL/mL gave an IZ value of 18.1%. The lowest IC_{50} values of 183 and 386 µL/mL were achieved by the application of eugenol and *p*-cymene, respectively, on wood (Table 4).

The highest FIPs observed against the growth of *Aspergillus niger* were 21.5% and 16.3%, by *p*-cymene and iso-eugenol, respectively, at 100 µL/mL, followed by iso-eugenol at 80 µL/mL (11.8%) and *p*-cymene at 60 µL/mL (18.1%), as shown in Table 3. The lowest IC_{50} values of 172 µL/mL and 202 µL/mL, were observed by the application of *p*-cymene and iso-eugenol, respectively, on wood (Table 5).

The highest FIPs observed against the growth of *Fusarium culmorum* were 41.5% and 27.0% by the application of carvacrol at 100 µL/mL and 80 µL/mL, respectively (Table 3), followed by eugenol at 100 µL/mL (23.7%). The lowest IC_{50} values of 129 7 and 155 µL/mL were reached by the application of carvacrol and iso-eugenol, respectively, on wood (Table 6).

Table 3. Antifungal Activity of Carvacrol, *p*-cymene, Eugenol, and Iso-eugenol Against *Aspergillus flavus*, *Aspergillus niger*, and *Fusarium culmorum*

Compound	Concentration	Fungal Inhibition Percentage (%)		
		Aspergillus flavus	*Aspergillus niger*	*Fusarium culmorum*
DMSO	10%	0.00g	0.00i	0.00o
Fluconazole	0.31%	19.62 ± 2.31bc	8.14 ± 1.28d	19.25 ± 2.79f
Carvacrol	20 µL/mL	0.00	0.00i	10.37 ± 0.64kl
	40 µL/mL	2.59 ± 0.64g	0.00i	12.96 ± 1.69ij
	60 µL/mL	5.18 ± 0.64f	0.00i	20.74 ± 1.28ef
	80 µL/mL	7.04 ± 0.64f	0.00i	27.04 ± 0.64b
	100 µL/mL	10.74 ± 0.64e	6.29 ± 0.64ef	41.48 ± 2.31a
p-Cymene	20 µL/mL	11.48 ± 0.64e	0.00i	8.52 ± 0.64l
	40 µL/mL	14.07 ± 1.28d	0.00i	11.48 ± 0.64jk
	60 µL/mL	18.14 ± 0.64c	5.92 ± 0.64ef	14.07 ± 1.28hi
	80 µL/mL	19.25 ± 1.28bc	11.48 ± 0.64c	17.037 ± 0.64g
	100 µL/mL	24.44 ± 2.22a	21.48 ± 1.28a	22.96 ± 1.28cd
Eugenol	20 µL/mL	0.00g	0.74 ± 0.64i	0.00o
	40 µL/mL	0.00g	2.59 ± 0.64h	4.81 ± 0.64m
	60 µL/mL	6.11 ± 0.78f	5.00 ± 0.78fg	10.55 ± 0.78kl
	80 µL/mL	9.72 ± 4.38e	6.66 ± 1.57e	13.05 ± 1.39ij
	100 µL/mL	21.11 ± 1.11b	11.48 ± 0.64c	23.71 ± 1.28c
Iso-eugenol	20 µL/mL	0.00g	1.48 ± 0.64hi	2.59 ± 0.64n
	40 µL/mL	0.00g	4.07 ± 0.64g	11.85 ± 0.64jk
	60 µL/mL	0.00g	7.03 ± 0.64de	15.92 ± 0.64gh
	80 µL/mL	17.04 ± 0.64c	11.85 ± 1.28c	21.48 ± 0.64de
	100 µL/mL	18.88 ± 1.11bc	16.29 ± 0.64b	22.59 ± 0.64cde

Means with the same letter are not significantly different according to Duncan's Multiple Range Test at 0.05 level of probability

Table 4. The IC_{50} Values Against the Growth of *Aspergillus flavus*

Tested compound	IC_{50} (µL/mL)[a]	Slope ± SE	Chi-test (χ2) Sig	95% CI		R^2
				Lower limit	Upper limit	
Carvacrol	541.581	1.721 ± 0.178	0.109	242.031	1211.871	0.965
p-Cymene	385.702	1.199 ± 0.182	0.903	169.935	875.427	0.951
Eugenol	183.011	3.285 ± 0.088	0.159	123.142	271.986	0.933
Iso-eugenol	1648.239	0.725 ± 0.305	-	415.611	6536.615	1.000

a: IC_{50}: Data expressed as µL/mL. Lower IC_{50} values indicate the highest antifungal activity.

Table 5. The IC_{50} Values Against the Growth of *Aspergillus niger*

Tested compound	IC_{50} (µL/mL)[a]	Slope ± SE	Chi-test (χ2) Sig	95% CI		R^2
				Lower limit	Upper limit	
Carvacrol	-	-	-	-	-	-
p-Cymene	172.497	3.450 ± 0.083	0.220	118.757	250.555	0.988
Eugenol	246.112	2.988 ± 0.110	0.649	149.507	405.139	0.947
Iso-eugenol	202.371	2.998 ± 0.096	0.785	131.037	312.538	0.974

a: IC_{50}: Data expressed as µL/mL. Lower IC_{50} values indicate the highest antifungal activity.

Table 6. The IC_{50} Values Against the Growth of *Fusarium culmorum*

Tested Compound	IC_{50} (µL/mL)[a]	Slope ± SE	Chi-test (χ2) Sig	95% CI		R^2
				Lower limit	Upper limit	
Carvacrol	128.836	2.504 ± 0.086	0.848	87.411	189.895	0.978
p-Cymene	316.074	1.523 ± 0.152	0.957	159.369	626.865	0.986
Eugenol	214.313	2.346 ± 0.109	0.030	131.321	349.754	0.871
Iso-eugenol	155.272	3.029 ± 0.082	0.151	107.142	225.023	0.865

a: IC_{50}: Data expressed as µL/mL. Lower IC_{50} values indicate the highest antifungal activity.

From the above results, the order of the bioactivity of monoterpenes against the growth of *Aspergillus flavus* was eugenol > *p*-cymene > carvacrol > Iso-eugenol; for the growth of *Aspergillus niger*, it was *p*-cymene > iso-eugenol > eugenol > carvacrol; and for *Fusarium culmorum*, it was carvacrol > iso-eugenol > eugenol > *p*-cymene.

Terpenoids, such as thymol and carvacrol are frequently present in the EO and play a significant role in its biological activity (Igoe *et al.* 1999; Hyldgaard *et al.* 2012). Excellent antimicrobial and anti-biofilm properties are exhibited by carvacrol, an intriguing bioactive substance that is active against a variety of Gram-positive and Gram-negative bacteria, fungi, and both planktonic and sessile human pathogens (Marchese *et al.* 2018).

Thymol, α-pinene, camphene, carvacrol, caryophyllene, myrcene, and α-terpineol—the principal constituents of *Thymus vulgaris* EO—were found to have strong antifungal activity against *Fusarium solani* (Abd-Ellatif *et al.* 2022). Carvacrol and thymol

are effective against foodborne microorganisms, including *Staphylococcus aureus* and *Salmonella* spp. (Arnal-Schnebelen *et al.* 2004; Casarin *et al.* 2016).

Carvacrol has been linked to antimicrobial properties due to its significant impact on the cytoplasmic membrane's structural and functional characteristics (Nostro and Papalia 2012). In comparison to carvacrol, the most hydrophobic compound, eugenol and menthol demonstrated less antimicrobial activity against the growth of various microorganisms, such as *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Lactobacillus plantarum*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, and one type of fungus, *Botrytis cinerea* (Ben Arfa *et al.* 2006).

It is worth mentioning that carvacrol could be included in various formulations for use in biomedical and food packaging applications, either by itself or in combination with one or more synergistic products (Nostro and Papalia 2012). The antifungal and antibiofilm properties of carvacrol and thymol are present (Memar *et al.* 2017). Because carvacrol has a free hydroxyl group, is hydrophobic, and has a phenol moiety, it has greater antimicrobial properties than other volatile chemicals found in EOs (Sharifi-Rad *et al.* 2018). The amount of carvacrol that prevented the growth of the foodborne pathogen *Bacillus cereus* on rice was dependent on the initial inoculum size, and concentrations of 0.15 mg/g and higher were found to be effective (Ultee *et al.* 2000). A synergistic interaction between the two chemicals was identified when carvacrol and cymene, another naturally occurring antimicrobial agent, were mixed (Miladi *et al.* 2017). This combination enhanced the antimicrobial capacity of carvacrol (Ultee *et al.* 2000).

These monoterpenes are found in several EOs from aromatic and medicinal plants, including ornamental plants, shrubs, and trees. The primary components of *Corymbia citriodora* leaves' EO were compounds citronellal, citronellol, and isopulegol. When applied to *Melia azedarach* wood, these compounds showed potential antifungal action against *Fusarium culmorum*, *Rhizoctonia solani*, and *Penicillium chrysogenum* (Behiry *et al.* 2020). *Mentha longifolia* EO containing carvacrol, 1,8-cineole, thymol, carvacryl acetate, and *p*-cymene (Patonay *et al.* 2021). Carvacrol, cinnamaldehyde, and geraniol are three antimicrobial substances that are thought to act quickly (Guimarães *et al.* 2019). The EOs of several plants, including wild bergamot (*Citrus aurantium bergamia*), pepperwort (*Lepidium flavum*), thyme (*Thymus vulgaris*), and oregano (*Origanum vulgare*), contain carvacrol (Sharifi-Rad *et al.* 2018).

The main component of clove and cinnamon leaves and buds is eugenol, a monoterpenoid that is categorized as a phenolic chemical and has the IUPAC designation 4-allyl-2-methoxy phenol (Cabral *et al.* 2013). Eugenol has an inhibiting impact on fungi. For example, Zhao *et al.* (2021) have shown that eugenol can significantly reduce the permeability of *Rhizoctonia solani*'s cell membrane by blocking the formation of ergosterol. This could prevent rice sheath blight. Additionally, it caused damage to the cellular membrane of *Botrytis cinerea*, which prevented it from growing (Olea *et al.* 2019).

In comparison to the other components in cinnamon oil, eugenol and cinnamaldehyde were found to have a greater impact on the growth of both brown and white rot fungi (wood decay fungi) (Chittenden and Singh 2011). These compounds were also found to be potential wood preservatives for the treatment of timber (Wang *et al.* 2005a; Geweely *et al.* 2024). Furthermore, it was discovered that the main component in Piper betle, eugenol, had greater potency as an inhibitor of fungal growth than the entire essential oil (Prakash *et al.* 2010). Eugenol was found to significantly suppress the growth of *Aspergillus* sp. and *Cladosporium* sp. (Abbaszadeh *et al.* 2014).

Zhang *et al.* (2016) documented the antifungal efficacy of pure monoterpenes, including but not limited to β-citronellol, carvacrol, citral, eugenol, geraniol, and thymol, against the fungal species *Trametes hirsuta*, *Schizophyllum commune*, and *Pycnoporus sanguineus*, which cause wood white-rot. The antifungal properties of essential oils from *Origanum vulgare*, *Cymbopogon citratus*, *Thymus vulgaris*, *Pelargonium graveolens*, *Cinnamomum zeylanicum*, and *Eugenia caryophyllata* were confirmed by Xie *et al.* (2017) against the wood-decaying fungi *T. hirsuta* and *Laetiporus sulphurous*. The most active compounds identified were carvacrol, citron, citronellol, cinnamaldehyde, eugenol, and thymol. It has been demonstrated that several common constituents of natural essential oils, including as eugenol, isoeugenol, (E)-2-methylcinnamic acid, α-methyl cinnamaldehyde, and cinnamaldehyde, efficiently prevent the growth of *L. sulphurous*, a brown-rot fungus, and *Lenzites betulina*, a white-rot fungus (Cheng *et al.* 2024). Wang *et al.* (2024), confirmed that the combination of eugenol and citral (CEC) has a substantial synergistic inhibitory impact on *Aspergillus niger*.

According to the findings of Reinprecht *et al.* (2019), out of five differing essential oils (basil, cinnamon, clove, oregano, and thyme), basil oil (which contains manly linalool) exhibited the strongest antifungal activity against the brown-rot fungus *Serpula lacrymans* and the white-rot fungus T. versicolor, while clove oil (which primarily contains eugenol) showed the lowest antifungal activity.

The EO from *Cupressus macrocarpa* leaves showed the presence of sabinene, 4-terpinenol, citronellol, citronellal, *p*-cymene, spathulenol, *γ*-terpinene, camphor, and limonene as main compounds. This EO showed the highest FIP (65.7% and 35.7%) against the growth of *F. solani* when applied to *P. sylvestris* sapwood at 50 and 25 mg/L, respectively (Mohareb *et al.* 2023). *Pinus roxburghii* Sarg. wood treated with 125 µL/mL of *Mentha longifolia* demonstrated inhibitory zone values of 21.3 mm against *A. niger* and 7.3 mm against *A. flavus*, respectively. Menthone and eucalyptol were identified by this EO as the main chemicals. Furthermore, the application of EOs from *M. longifolia* and *Citrus reticulata* at 500 µL/mL inhibited the growth of *F. culmorum* (100% FIP) (Ali *et al.* 2021). After being dipped in the EO made from *Origanum majorana* leaves, wood samples from *Acacia saligna*, *F. sylvatica*, *Juglans nigra*, and *P. rigida* showed strong antifungal effects against *A. niger* and *Trichoderma harzianum* without altering the wood's structural integrity (Salem *et al.* 2019).

The mechanism of action of monoterpenes has been shown in specific investigations to cause the breakdown of cytoplasmic and organelle membranes. Changes in membrane function based on a lack of membrane integrity may result in antifungal activity (Sikkema *et al.* 1995; Pinto *et al.* 2006; Park *et al.* 2009). The hyphae of *Trichophyton mentagrophytes* were distorted and collapsed at 0.2, 0.4 and 1 mg/mL of eugenol, nerolidol and α-terpineol, respectively (Park *et al.* 2009). Eugenol was found to play a greater role than citral in altering cell membrane morphology of fungi (Wang *et al.* 2024). The *Zygosaccharomyces rouxii* surface morphological folding or deformation is caused by both eugenol and citral, according to a previous observation. According to Cai *et al.* (2019), the eugenol group exhibited a higher degree of cell wrinkling in comparison to the citral group.

CONCLUSIONS

1. Four monoterpenes, namely carvacrol, *p*-cymene, eugenol, and iso-eugenol, were used as antifungal agents when applied to wood against *Aspergillus flavus*, *Aspergillus niger*, and *Fusarium culmorum*.
2. Carvacrol at 100 µL/mL with *F. culmorum*, *p*-cymene at 100 µL/mL with *A. flavus* and *F. culmorum*, and eugenol and iso-eugenol at 100 µL/mL with *F. culmorum*.
3. The lowest IC_{50} values (the concentration of a compound required to inhibit the fungal growth by 50%) of 183 and 386 µL/mL against the growth of *A. flavus* were observed by the application of eugenol and *p*-cymene, respectively, on wood.
4. When *p*-cymene and iso-eugenol were applied to wood, the lowest IC_{50} values of 172 and 202 µL/mL, respectively, were noted against the development of *A. niger*.
5. The lowest IC_{50} values of 129 and 155 µL/mL were observed against the growth of *F. culmorum* by the application of carvacrol and iso-eugenol, respectively, on wood.

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