Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

Broad-Spectrum Antimicrobial Activity of *Thymus zygis* Essential Oil Against Various Microorganisms

Dilek Kahraman Yılmaz[a]

[a]*Department of Marine Biology, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, Çanakkale 17100, TÜRKİYE*

ARTICLE INFO
Research Article
Corresponding Author: Dilek Kahraman Yılmaz, E-mail: dilek.kahramanyilmaz@comu.edu.tr
Received: 05 May 2025 / Revised: 15 August 2025 / Accepted: 25 September 2025 / Online: 20 January 2026

Cite this article
Kahraman Yılmaz D (2026). Broad-Spectrum Antimicrobial Activity of *Thymus zygis* Essential Oil Against Various Microorganisms. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 32(1):177-185. DOI: 10.15832/ankutbd.1691636

ABSTRACT
In this study, the antimicrobial effect of the *T. zygis* essential oil (EO) was investigated on 68 bacterial and two yeast isolates belonging to 20 different families, including foodborne, ready-to-eat food, aquatic, and collection strains that are important for food safety and aquaculture. First, the chemical composition of the oil was analyzed using GC-MS to better understand its potential bioactivity. The major components of the *T. zygis* EO were identified as thymol (38.82%), β-cymene (21.66%), γ-terpinene (6.51%), carvacrol (6.36%) and linalool (6.25%) and the total of these components constituted 79.6% of the essential oil. In terms of antimicrobial activity, the disk diffusion test revealed inhibition in 66 out of 70 tested strains, with strong inhibition observed in 62 strains, moderate inhibition in four, and no inhibition in the remaining four. It showed strong antimicrobial activity against species known to be foodborne and fish pathogens such as *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Escherichia coli*, *Pseudomonas putida*, *Salmonella enterica*, *Staphylococcus aureus*, *Streptococcus iniae*, *Klebsiella pneumoniae*, *Edwardsiella tarda*, *Lactococcus garvieae*, *Yersinia ruckeri*, *Vibrio parahaemolyticus*, *Vibrio anguillarum*, and *Vibrio gigantis*. In addition, it has been determined that the *T. zygis* EO has strong antifungal activity against yeast species such as *Candida albicans* and *Saccharomyces cerevisiae*. Antimicrobial activity levels were attributed to the high levels of phenolic components, especially thymol. In conclusion, this study has shown that the essential oil derived from *T. zygis* exhibits broad-spectrum antimicrobial activity and an effective natural bioprotective agent for both Gram-positive and Gram-negative bacteria and yeast species. In addition, this study provides an original contribution to the literature through a comprehensive evaluation of its effect on microorganisms isolated from various sources. Future studies should focus on the stability, sensory properties, and mechanisms of action of the *T. zygis* essential oil, particularly in diverse seafood applications.

Keywords: Thyme, Antimicrobial Components, Bioprotective, Food Pathogens, Fish Isolate

1. Introduction

In recent years, interest in natural alternatives to control pathogenic microorganisms that threaten food safety and aquaculture health has increased. Foodborne pathogens and bacteria that cause fish diseases pose a serious risk to human health and the aquaculture industry (Kahraman Yılmaz & Berik 2024; Kahraman Yılmaz & Berik 2025). Bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Vibrio parahaemolyticus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* spp., and *Aeromonas hydrophila*, which are commonly seen in foods and aquaculture systems, cause not only food spoilage but also the spread of diseases (Janda & Abbott 2010; Defoirdt et al. 2011; Defoirdt 2014; Olaimat et al. 2024). In particular, biofilm formation enables some bacteria to resist disinfectants, making food safety and infection control in aquaculture challenging (Tomás 2012; Arthur et al. 2025). The resistance of these microorganisms to classical antibiotic treatments has necessitated the search for natural and safe alternatives instead of synthetic preservatives (Wu et al. 2024; Elafify et al. 2025). In this direction, herbal essential oils, known for their antimicrobial, antioxidant, and antibiofilm properties, have been intensively researched in recent years (Farrukh et al. 2025). Essential oils obtained from aromatic plants are rich in bioactive compounds such as phenolic compounds, terpenoids, and flavonoids. They have broad-spectrum antimicrobial potential (Othman et al. 2019; Šojić et al. 2023).

T. zygis is a medicinal and aromatic plant belonging to the Lamiaceae family and native to the Mediterranean region. It contains strong antimicrobial components such as thymol, carvacrol, cymene, terpinene, and linalool (Ziegler 2007). The U.S. Food and Drug Administration (FDA) lists *T. zygis* essential oil as generally recognized as safe (GRAS) for use in foods, in accordance with its intended use under 21 CFR section 182.20 (Electronic Code of Federal Regulations 2025).

Various studies have reported that *T. zygis* essential oil is effective against both Gram-positive and Gram-negative bacteria and also has an inhibitory effect on fungal species (Pina-Vaz et al. 2004; Radi et al. 2022; Vukić et al. 2023; Aabouch et al. 2024).

In the literature, the antimicrobial effects of *T. zygis* on microorganisms that cause spoilage in food have been widely investigated. However, studies on its effects on microorganisms isolated from fish and some collection strains are limited (Coimbra et al. 2022-Review article), and comprehensive assessments against a wide microbial spectrum from diverse sources are lacking. In particular, comprehensive assessments against the wide microbial spectrum obtained from different isolation sources appear to be lacking. In this study, microorganisms whose antimicrobial effects have not been previously evaluated with *T. zygis* essential oil in the literature include *Aeromonas salmonicida*, *Aeromonas sobria*, *Aeromonas veronii*, *Shewanella baltica*, *Bacillus thuringiensis*, *Bacillus pumilus*, *Bacillus licheniformis*, *Lysinibacillus xylanilyticus*, *Niallia nealsonii*, *Staphylococcus epidermidis*, *Staphylococcus equorum*, *Staphylococcus haemolyticus*, *Staphylococcus pasteuri*, *Staphylococcus warneri*, *Lelliottia amnigena*, *Plesiomonas shigelloides*, *Serratia grimesii*, *Serratia liquefaciens*, *Serratia proteamaculans*, *Edwardsiella tarda*, *Yersinia ruckeri*, *Flavobacterium tructae*, *Chryseobacterium* spp., *Aerococcus viridans*, *Carnobacterium maltaromaticum*, *Carnobacterium* sp., *Kocuria rhizophila*, *Acinetobacter johnsonii*, *Psychrobacter* sp., *Vibrio gigantis*, *V. anguillarum*, *V. hibernica*, *V. rumoiensis*, *Lactococcus garvieae*, *Streptococcus iniae* and *Saccharomyces cerevisiae*.

This study hypothesizes that the essential oil from *T. zygis*, due to its high phenolic content, will exhibit potent antimicrobial activity against a wide range of microorganisms, including fish- and foodborne pathogens, many of which have not been previously evaluated. The broad range of isolates tested in this study was carefully selected to assess the oil's potential as a broad-spectrum natural bioprotective agent for both food safety and aquaculture applications.

2. Material and Methods

2.1. Essential oil and chemical composition analysis

The *T. zygis* essential oil used in this study was obtained from a commercial supplier (Tito, Smart Kimya, İzmir, Türkiye). The chemical composition of the essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS), following the protocol described in our previous study (Kahraman Yılmaz & Berik 2023), after dilution with n-hexane. A Shimadzu GCMS QP 2010 ULTRA equipped with an Rxi-5 ms capillary column was used, with helium as the carrier gas. The obtained chromatograms were compared with the Wiley W9N11 mass spectral library for the identification of the components.

2.2. Bacterial material

In this study, the antimicrobial effect of *T. zygis* essential oil was investigated against a total of 70 isolates, including 68 bacteria and two yeast isolates, belonging to 20 different families. Specifically, the antimicrobial activity was evaluated for Gram-negative bacteria (Table 1) as well as Gram-positive bacteria and yeasts (Table 2). All strains were maintained as stock cultures at -80 °C in the Ecotoxicology Research Laboratory of the Faculty of Marine Sciences and Technology of Çanakkale Onsekiz Mart University.

Table 1- Gram-negative bacterial isolates used in the antimicrobial activity tests, with taxonomic classification, strain codes, GenBank/collection numbers, and isolation sources

Family	***Genus/Species and Strain Code***	***GenBank/Collection No***	***Isolation Source***
Aeromonadaceae	*Aeromonas hydrophila* SY-AH2	MG844996.1	Fish isolate
Aeromonadaceae	*Aeromonas salmonicida* subsp. *salmonicida* ATCC 33658	-	Collection strain
Aeromonadaceae	*Aeromonas sobria* SY-AS1	KY126835.1	Fish isolate
Aeromonadaceae	*Aeromonas sobria* SY-AS3	KY126831.1	Fish isolate
Aeromonadaceae	*Aeromonas veronii* SY-AV10	MG563680.1	Fish isolate
Shewanellaceae	*Shewanella baltica* SY-S145	KX388237.1	Fish isolate
Enterobacteriaceae	*Citrobacter freundii* SY-CF14	KY126840.1	Fish isolate
Enterobacteriaceae	*Citrobacter* sp. SY-C10	KX388233.1	Fish isolate
Enterobacteriaceae	*Enterobacter* sp. SY-EN560	KX388232.1	Fish isolate
Enterobacteriaceae	*Escherichia coli* ATCC 25922	-	Collection strain
Enterobacteriaceae	*Escherichia coli* SY-EC4	MG855664.1	Fish isolate
Enterobacteriaceae	*Klebsiella pneumoniae* ATCC BAA-1144	-	Collection strain
Enterobacteriaceae	*Klebsiella pneumoniae* SY-KP1	MG855663.1	Fish isolate
Enterobacteriaceae	*Lelliottia amnigena* SY-LA18	KY126834.1	Fish isolate
Enterobacteriaceae	*Plesiomonas shigelloides* SY-PS16	MG574356.1	Fish isolate
Enterobacteriaceae	*Salmonella enterica* serovar Enteritidis ATCC 13076	-	Collection strain
Enterobacteriaceae	*Serratia grimesii* KY-SS7	PV459074.1	Seafood salad
Enterobacteriaceae	*Serratia grimesii* KY-SS9	PV457913.1	Seafood salad
Enterobacteriaceae	*Serratia liquefaciens* DKY-SS10	PV458288.1	Seafood salad
Enterobacteriaceae	*Serratia liquefaciens* DKY-SS3	PV459209.1	Seafood salad
Enterobacteriaceae	*Serratia proteamaculans* DKY-SS16	PV459206.1	Seafood salad
Enterobacteriaceae	*Serratia proteamaculans* DKY-SS17	PV459208.1	Seafood salad
Enterobacteriaceae	*Serratia* sp. DKY-SS11	PV458349.1	Seafood salad
Hafniaceae	*Edwardsiella tarda* SY-ED14	KX388234.1	Fish isolate
Hafniaceae	*Edwardsiella tarda* SY-ED1	KY126838.1	Fish isolate
Yersiniaceae	*Yersinia ruckeri* E42	KX388238.1	Fish isolate
Flavobacteriaceae	*Chryseobacterium* sp. DKY-AA281	PV583358.1	Salt-cured sardines
Flavobacteriaceae	*Chryseobacterium* sp. DKY-UYA270	PV583221.1	Salt-cured sardines
Flavobacteriaceae	*Flavobacterium tructae* SY-FS1	KY386294.1	Fish isolate
Moraxellaceae	*Acinetobacter johnsonii* SY-AJ	KY126836.1	Fish isolate
Moraxellaceae	*Psychrobacter* sp. DKY-UYA1	PV582990.1	Salt-cured sardines
Pseudomonadaceae	*Pseudomonas aeruginosa* ATCC 10145	-	Collection strain
Pseudomonadaceae	*Pseudomonas putida* SY-PP21	KY126830.1	Fish isolate
Pseudomonadaceae	*Pseudomonas* sp. DKY-KIL2	PV583363.1	Lakerda
Vibrionaceae	*Vibrio parahaemolyticus* ATCC 17802	-	Collection strain
Vibrionaceae	*Vibrio anguillarum* SY-L24	KX388236.1	Fish isolate
Vibrionaceae	*Vibrio gigantis* C24	ON778781.1 / ON792326.1	Fish isolate
Vibrionaceae	*Vibrio hibernica* DKY-ML304	PV582992.1	Lakerda
Vibrionaceae	*Vibrio rumoiensis* DKY-ML248	PV575816.1	Lakerda

Table 2- Gram-positive bacterial isolates and yeast strains used in the antimicrobial activity tests, with taxonomic classification, strain codes, GenBank/collection numbers, and isolation sources

Family	*Genus/Species and Strain Code*	*GenBank/Collection No*	*Isolation Source*
Bacillaceae	*Bacillus subtilis* SY-BS19	KY077683.1	Fish isolate
Bacillaceae	*Bacillus subtilis* SY-BS20	KY078783.1	Fish isolate
Bacillaceae	*Bacillus thuringiensis* SY-BT11	KY127450.1	Fish isolate
Bacillaceae	*Bacillus licheniformis* DKY-AA247	PV583360.1	Salt-cured sardines
Bacillaceae	*Bacillus pumilus* SY-BP15	KY126833.1	Fish isolate
Bacillaceae	*Bacillus* sp. DKY-ENG173	PV583354.1	Salt-cured sardines
Bacillaceae	*Bacillus* sp. DKY-YAK186	PV583222.1	Salt-cured sardines
Bacillaceae	*Bacillus spizizenii* ATCC 6633	-	Collection strain
Bacillaceae	*Lysinibacillus xylanilyticus* SY-LX12	KY078822.1	Fish isolate
Bacillaceae	*Niallia nealsonii* SY-BN9	KY126832.1	Fish isolate
Staphylococcaceae	*Staphylococcus aureus* ATCC 25923	-	Collection strain
Staphylococcaceae	*Staphylococcus epidermidis* DKY-A10	PV575643.1	Salt-cured sardines
Staphylococcaceae	*Staphylococcus epidermidis* DKY-SS1	PV459212.1	Seafood salad
Staphylococcaceae	*Staphylococcus epidermidis* DKY-YA235	PV575815.1	Salt-cured sardines
Staphylococcaceae	*Staphylococcus equorum* DKY-BL3	PV575741.1	Lakerda
Staphylococcaceae	*Staphylococcus haemolyticus* DKY-UYL97	PV583349.1	Lakerda
Staphylococcaceae	*Staphylococcus haemolyticus* DKY-YAK205	PV575817.1	Salt-cured sardines
Staphylococcaceae	*Staphylococcus pasteuri* DKY-M16	PV405943.1	Stuffed mussel
Staphylococcaceae	*Staphylococcus pasteuri* DKY-UYL2	PV575805.1	Lakerda
Staphylococcaceae	*Staphylococcus warneri* DKY-M10	PV405942.1	Stuffed mussel
Staphylococcaceae	*Staphylococcus warneri* DKY-M12	PV405944.1	Stuffed mussel
Aerococcaceae	*Aerococcus viridans* DKY-M1	PV396891.1	Stuffed mussel
Carnobacteriaceae	*Carnobacterium maltaromaticum* DKY-KOC29	PV583362.1	Salt-cured sardines
Carnobacteriaceae	*Carnobacterium maltaromaticum* DKY-KOC60	PV583364.1	Salt-cured sardines
Carnobacteriaceae	*Carnobacterium* sp. DKY-L10	PV583024.1	Lakerda
Lactobacillaceae	*Lactiplantibacillus plantarum* BC 7321	-	Collection strain
Streptococcaceae	*Lactococcus garvieae* SY-LG1	KY118086.1	Fish isolate
Streptococcaceae	*Streptococcus iniae* ATCC 29177	-	Collection strain
Micrococcaceae	*Kocuria rhizophila* DKY-UYA3	PV575796.1	Salt-cured sardines
Saccharomycetaceae	*Candida albicans* ATCC 10231	-	Collection strain
Saccharomycetaceae	*Saccharomyces cerevisiae* ATCC 18824	-	Collection strain

2.3. Disk diffusion test

The antibacterial activity of *T. zygis* essential oil against different bacterial and yeast isolates was evaluated using the disk diffusion method (Bauer et al. 1966). For this purpose, 10 µL of *T. zygis* essential oil was impregnated onto sterile blank disks (Oxoid, CT0998B) and dried in sterile 96-well plates under aseptic conditions. Since the essential oil was applied directly without any solvent, sterile blank disks without any treatment were used as negative controls. The disks were placed on inoculated agar plates using sterile forceps, and the plates were incubated at the appropriate temperatures for 24–36 hours. The diameters of the inhibition zones formed after incubation were measured; zones of ≥20 mm were considered strong inhibition, zones of 12–19 mm moderate inhibition, and zones of <12 mm ineffective (Rota et al. 2008).

3. Results and Discussions

3.1. Chemical composition of Thymus zygis essential oil

In this study, among the 32 components identified in *T. zygis* essential oil (Table 3), thymol (38.82%), *β*-cymene (21.66%), *γ*-terpinene (6.51%), carvacrol (6.36%), and linalool (6.25%) together constituted 79.60% of the essential oil.

Table 3- Chemical composition of *Thymus zygis* essential oil obtained by GC-MS analysis

Peak	*Retention Time*	*Area%*	*Components*
1	9.331	1.05	alpha-Thujene
2	9.559	2.47	alpha-Pinene
3	10.115	0.54	Camphene
4	11.242	2.51	beta-Pinene
5	11.787	0.07	Cyclohexane, 1-methyl-4-(1-methylethyl)-, cis-
6	11.952	2.43	Myrcene
7	12.375	0.16	Pseudolimonene
8	12.622	0.03	delta 3-Carene
9	12.915	1.36	alpha-Terpinene
10	13.105	0.04	Cyclohexene, 1-methyl-4-(1-methylethyl)-, (R)-
11	13.164	0.14	o-Cymene
12	13.317	21.66	β-cymene
13	13.423	2.03	Limonene
14	13.489	0.60	Eucalyptol
15	14.650	6.51	gamma-Terpinene
16	15.090	0.12	trans Sabinene hydrate
17	15.234	0.06	trans-Linalool oxide
18	15.793	0.05	alpha-Terpinolene
19	15.866	0.07	trans-Linalool oxide
20	16.337	0.03	trans Sabinene hydrate
21	16.473	6.25	linalool
22	18.404	0.15	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2R-cis)-
23	18.884	0.02	Ethanone, 1-(1,4-dimethyl-3-cyclohexen-1-yl)- (
24	19.024	1.17	endo-Borneol
25	19.220	0.04	2-Cyclohexen-1-one, 5-methyl-2-(1-methylethyl)-
26	19.419	2.34	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-
27	21.860	0.59	Carvacrol methyl ether
28	23.462	0.04	Anethole
29	24.235	38.82	Thymol
30	24.511	6.36	Carvacrol
31	28.057	1.49	Caryophyllene
32	33.366	0.80	Caryophyllene oxide

These findings are largely in agreement with the major components specified for *T. zygis*, namely thymol (37–55%), carvacrol (0.5–5.5%), *p*-cymene (14–28%), *γ*-terpinene (4–11%) and linalool (3–6.5%) in the ISO 14715:1999 standard (International Organization for Standardization 1999). Similarly, for *T. zygis* essential oil, Pina-Vaz et al. (2004) reported 39.6% thymol, 21.2% *p*-cymene, 7.9% *γ*-terpinene and 5.5% linalool. Dorman & Deans (2004) also identified thymol (48.0%) and *p*-cymene (25.5%) as the most dominant components. However, Aabouch et al. (2024) reported a different composition for *T. zygis* essential oil, with *δ*-terpineol (27.64%), *δ*-3-carene (15.70%), and thymol (14.17%) predominating. Another study found a remarkable difference. Among the total 31 components, thymol was found only at 9.68%, while carvacrol (52.20%) stood out as the main compound (Radi et al. 2022). In the study conducted by Vukić et al. (2022), 59 components were identified, and linalool (38.0%) was determined as the most dominant component instead of thymol, followed by monoterpene components such as 4-terpineol (10.1%), *p*-cymene (6.9%) and *γ*-terpinene (6.5%).

This indicates that the component ratios in *T. zygis* samples can vary significantly depending on environmental variables such as geographical conditions, ecological factors, and harvest time. In fact, in the studies conducted by Morales (1986) and Sáez (1995) on *T. zygis* species naturally growing in the southeast of Spain, it was reported that the most common chemotype in this species was thymol, but pure linalool chemotype was also found. This may explain the high rate of linalool in some studies (Rota et al. 2008). Variation in the chemical composition of *T. zygis* essential oil, particularly the proportions of thymol, *p*-cymene, carvacrol, and linalool, can lead to significant differences in antimicrobial activity. Because the biological activity of the essential oil is concentration-dependent, higher levels of phenolic monoterpenes such as thymol and carvacrol are generally associated with stronger inhibition by disrupting the cell membrane and increasing permeability (Joshi et al. 2011; Chouhan et al. 2017). Conversely, lower concentrations of these compounds or a shift to less active compounds may reduce efficacy. Such compositional differences may be influenced by environmental factors, harvesting period, and storage conditions and should be considered when comparing results between studies.

3.2. Antimicrobial test results

T. zygis essential oil inhibited 66 (~94%) of the 70 tested isolates, showing strong inhibition in 62 and moderate inhibition in 4 strains. All four non-inhibited strains were Gram-negative bacteria (Table 4), whereas all Gram-positive bacteria and yeasts were inhibited (Table 5).

Table 4- Antimicrobial effect of *T. zygis* essential oil on Gram-negative bacterial strains

Genus/Species and Strain Code	*Test Media*	*Incubation Temperature (°C)*	*Zone**	*Result*
Aeromonas hydrophila SY-AH2	MHA	35	42	SI
Aeromonas salmonicida subsp. *salmonicida* ATCC 33658	MHA	24	60	SI
Aeromonas sobria SY-AS1	MHA	28	47	SI
Aeromonas sobria SY-AS3	MHA	28	62	SI
Aeromonas veronii SY-AV10	MHA	35	38	SI
Shewanella baltica SY-S145	MHA+1.5% NaCI	22	40	SI
Citrobacter freundii SY-CF14	MHA	28	13	MI
Citrobacter sp. SY-C10	MHA	28	16	MI
Enterobacter sp. SY-EN560	MHA	28	34	SI
Escherichia coli ATCC 25922	MHA	35	22	SI
Escherichia coli SY-EC4	MHA	35	24	SI
Klebsiella pneumoniae ATCC BAA-1144	MHA	35	18	MI
Klebsiella pneumoniae SY-KP1	MHA	35	13	MI
Lelliottia amnigena SY-LA18	MHA	35	35	SI
Plesiomonas shigelloides SY-PS16	MHA	35	48	SI
Salmonella enterica serovar Enteritidis ATCC 13076	MHA	35	22	SI
Serratia grimesii KY-SS7	MHA	35	36	SI
Serratia grimesii KY-SS9	MHA	35	30	SI
Serratia liquefaciens DKY-SS10	MHA	35	10	NI
Serratia liquefaciens DKY-SS3	MHA	35	10	NI
Serratia proteamaculans DKY-SS16	MHA	35	27	SI
Serratia proteamaculans DKY-SS17	MHA	35	28	SI
Serratia sp. DKY-SS11	MHA	35	30	SI
Edwardsiella tarda SY-ED14	MHA	28	54	SI
Edwardsiella tarda SY-ED1	MHA	28	58	SI
Yersinia ruckeri E42	MHA	24	41	SI
Chryseobacterium sp. DKY-AA281	MHA	22	80	SI
Chryseobacterium sp. DKY-UYA270	MHA	22	80	SI
Flavobacterium tructae SY-FS1	TSA	22	35	SI
Kocuria rhizophila DKY-UYA3	MHA	22	30	SI
Acinetobacter johnsonii SY-AJ	MHA	22	34	SI
Psychrobacter sp. DKY-UYA1	MHA	24	11	NI
Pseudomonas aeruginosa ATCC 10145	MHA	35	10	NI
Pseudomonas putida SY-PP21	MHA	22	43	SI
Pseudomonas sp. DKY-KIL2	MHA	24	34	SI
Vibrio parahaemolyticus ATCC 17802	MHA+1.5% NaCI	24	42	SI
Vibrio anguillarum SY-L24	MHA+1.5% NaCI	24	40	SI
Vibrio gigantis C24	MHA+1.5% NaCI	24	30	SI
Vibrio hibernica DKY-ML304	MHA+1.5% NaCI	24	42	SI
Vibrio rumoiensis DKY-ML248	MHA+1.5% NaCI	24	50	SI

* Zone diameter including disk (mm). MHA: Mueller-Hinton agar; TS: tryptic soy agar; BHA: brain heart infusion agar; MRS: de Man, Rogosa and Sharpe agar; SI: Strong inhibition (≥20 mm), MI: Moderate inhibition (12–19 mm), NI: No inhibition (<12 mm)

Table 5- Antimicrobial effect of *T. zygis* essential oil on Gram-positive bacterial and yeast strains

Genus/Species and Strain Code	*Test Media*	*Incubation Temperature (°C)*	*Zone**	*Result*
Bacillus subtilis SY-BS19	MHA	35	48	SI
Bacillus subtilis SY-BS20	MHA	35	39	SI
Bacillus thuringiensis SY-BT11	MHA	35	36	SI
Bacillus licheniformis DKY-AA247	MHA	35	49	SI
Bacillus pumilus SY-BP15	MHA	35	39	SI
Bacillus sp. DKY-ENG173	MHA	35	38	SI
Bacillus sp. DKY-YAK186	MHA	35	40	SI
Bacillus spizizenii ATCC 6633	MHA	35	38	SI
Lysinibacillus xylanilyticus SY-LX12	MHA	35	36	SI
Niallia nealsonii SY-BN9	TSA	35	46	SI
Staphylococcus aureus ATCC 25923	MHA	35	25	SI
Staphylococcus epidermidis DKY-A10	MHA	35	40	SI
Staphylococcus epidermidis DKY-SS1	MHA	35	35	SI
Staphylococcus epidermidis DKY-YA235	MHA	35	32	SI
Staphylococcus equorum DKY-BL3	MHA	35	26	SI
Staphylococcus haemolyticus DKY-UYL97	MHA	35	45	SI
Staphylococcus haemolyticus DKY-YAK205	MHA	35	40	SI
Staphylococcus pasteuri DKY-M16	MHA	35	28	SI
Staphylococcus pasteuri DKY-UYL2	MHA	35	35	SI
Staphylococcus warneri DKY-M10	MHA	35	25	SI
Staphylococcus warneri DKY-M12	MHA	35	25	SI
Aerococcus viridans DKY-M1	MHA	35	30	SI
Carnobacterium maltaromaticum DKY-KOC29	MHA	22	50	SI
Carnobacterium maltaromaticum DKY-KOC60	MHA	22	31	SI
Carnobacterium sp. DKY-L10	MHA	22	80	SI
Lactiplantibacillus plantarum BC 7321	MRS	30	20	SI
Lactococcus garvieae SY-LG1	MHA	24	35	SI
Streptococcus iniae ATCC 29177	BHI+5% Blood	35	26	SI
Candida albicans ATCC 10231	MHA+2% glucose	24	36	SI
Saccharomyces cerevisiae ATCC 18824	MHA+2% glucose	24	43	SI

* Zone diameter including disk (mm). MHA: Mueller-Hinton agar; TS: tryptic soy agar; BHA: brain heart infusion agar; MRS: de Man, Rogosa and Sharpe agar; SI: Strong inhibition (≥20 mm), MI: Moderate inhibition (12–19 mm), NI: No inhibition (<12 mm)

The findings showed that antimicrobial activity was observed against important pathogenic and spoilage bacteria of fish and food origin, such as *Aeromonas*, *Bacillus*, *Staphylococcus*, *Edwardsiella*, *Pseudomonas*, *Vibrio*. These results are consistent with previous studies (Dorman & Deans 2004; Pina-Vaz et al. 2004; Radi et al. 2022; Vukić et al. 2023; Aabouch et al. 2024), but unlike earlier work, this study evaluated the essential oil against a wider range of microorganisms from fish, seafood, food, and culture collections. For example, *T. zygis* essential oil exhibited a strong inhibitory effect on *A. hydrophila*, *B. subtilis*, *E. coli*, *K. pneumoniae*, *L. plantarum* and *P. aeruginosa* (Dorman & Deans 2004). Similarly, it produced large inhibition zones against *Listeria monocytogenes* serotypes 1/2c (44.0 mm) and 4b (36.6 mm), *Yersinia enterocolitica* (46.6 mm), *Shigella flexneri* (36.0 mm), *Salmonella enteritidis* (32.2 mm), *S. typhimurium* (30.3 mm), *S. sonnei* (30.0 mm), *S. aureus* (25.0 mm), *E. coli* O157:H7 (20.3 mm) and *E. coli* (16.0 mm) (Rota et al. 2008). In the study of Vukić et al. (2022) the essential oil inhibited Gram-positive (*Listeria monocytogenes*, *Micrococcus luteus*, *Staphylococcus aureus*), Gram-negative (*Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas putida*) and four yeast species (*Candida albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*). Similarly, Radi et al. (2022) reported strong antibacterial activity against *S. aureus*, *E. coli*, *Salmonella Typhi*, *Acinetobacter baumannii*, *Enterobacter cloacae* and *Shigella dysenteriae*, and antifungal effects against *C. albicans*, *Candida sp.*, *Candida glabrata*, *Aspergillus fischeri* and *Fusarium solani*. In contrast, Aabouch et al. (2024) found smaller inhibition zones (17.11-18.33) for *B. subtilis* (ATCC 6633), *S. aureus* (ATCC 6538), and *E. coli* (ATCC 8739), likely due to the use of 5 µL/disc versus 10 µL/disc in this study.

The potent antimicrobial activity of *T. zygis* essential oil can be attributed to its main phenolic components, particularly thymol and carvacrol. Thymol disrupts the lipid bilayer of microbial cell membranes, increasing permeability and leading to leakage of cell contents (Burt 2004; Marchese et al. 2016; Chouhan et al. 2017). Carvacrol similarly integrates into the lipid matrix, affecting membrane proteins, altering the proton motive force and ultimately leading to ATP depletion (Pei et al. 2009; Joshi et al. 2011; Touati et al. 2025). Monoterpene alcohols, namely linalool, have also been reported to alter membrane fluidity and increase ion

permeability (Joshi et al. 2011). Beyond their direct bactericidal effects, these components can suppress biofilm formation by inhibiting quorum sensing pathways and reduce virulence factor production (Kerekes et al. 2013). However, despite its weak antimicrobial properties, β-cymene (21.66%), present in high concentrations, can localize to the cell membrane, leading to membrane expansion and potential changes (Hyldgaard et al. 2012). This property has been reported to provide a synergistic effect by facilitating more efficient entry of compounds such as thymol and carvacrol into the cell (Hyldgaard et al. 2012; Touati et al. 2025). These multifaceted mechanisms likely explain the broad-spectrum antimicrobial effect observed in this study.

4. Conclusions

This study demonstrated that *T. zygis* essential oil exhibits broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria and yeast species due to its rich phenolic content. The high inhibitory activity it exhibits on pathogenic, non-pathogenic, and collection microorganisms isolated from food and aquatic products supports the potential of this essential oil as a natural preservative. These findings obtained on many fish and food isolates tested for the first time, provide a new perspective for the evaluation of *T. zygis* essential oil as a natural antimicrobial agent, especially in food safety and aquaculture. *T. zygis* EO is generally recognized as safe (GRAS) for its intended use in foods under 21 CFR section 182.20. Therefore, its application in food systems is permitted from a toxicological and regulatory perspective; however, sensory effects on the final product must be evaluated to ensure consumer acceptance. Future studies should examine in detail the stability, sensory effects, effective dosage ranges, and antimicrobial mechanisms of this oil in different applications in the seafood sector.

Author Declarations

Authors' contributions: Concept: D.K.Y, Design: D.K.Y, Data collection: D.K.Y, Analysis: D.K.Y, Writing: D.K.Y.

Conflict of interest/Competing interests: The author(s) declare no conflict of interest.

Ethics approval/declarations: No need/ Not applicable.

Funding: This research received no external funding.

Consent to participate: No need/ Not applicable.

Consent for publication: No need/ Not applicable.

Data availability: Data are available on request due to privacy or other restrictions.

References

Aabouch F, Annemer S, Satrani B, Ettaleb I, Kara M, Ghanmi M Shahat A A, Choudhary R, Farah A, Ouajdi M & Dahmani J (2024). Assessing the Optimal Antibacterial Action of *Lavandula stoechas* L., *Thymus zygis* L., and *Eucalyptus camaldulensis* Dehnh Essential Oils. *Life* 14(11): 1424. https://doi.org/10.3390/life14111424

Arthur M, Afari E L, Alexa E A, Zhu M J, Gaffney M T, Celayeta J M F & Burgess C M (2025). Recent advances in examining the factors influencing the efficacy of biocides against *Listeria monocytogenes* biofilms in the food industry: A systematic review. *Comprehensive Reviews in Food Science and Food Safety* 24(1): e70083. https://doi.org/10.1111/1541-4337.70083

Burt S (2004). Essential oils: Their antibacterial properties and potential applications in foods—A review. *International Journal of Food Microbiology* 94(3): 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Chouhan S, Sharma K & Guleria S (2017). Antimicrobial activity of some essential oils—present status and future perspectives. *Medicines* 4(3): 58. https://doi.org/10.3390/medicines4030058

Coimbra A, Ferreira S & Duarte A P (2022). Biological properties of *Thymus zygis* essential oil with emphasis on antimicrobial activity and food application. *Food Chemistry* 393: 133370. https://doi.org/10.1016/j.foodchem.2022.133370

Defoirdt T (2014). Virulence mechanisms of bacterial aquaculture pathogens and antivirulence therapy for aquaculture. *Reviews in Aquaculture* 6(2): 100-114. https://doi.org/10.1111/raq.12030

Defoirdt T, Sorgeloos P & Bossier P (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Current Opinion in Microbiology* 14(3): 251–258. https://doi.org/10.1016/j.mib.2011.03.004

Dorman H J D & Deans S G (2004). Chemical composition, antimicrobial and in vitro antioxidant properties of *Monarda citriodora*, *Marjorana fragrans*, *Origanum vulgare*, *Pelargonium* species and *Thymus zygis* oils. *Journal of Essential Oil Research* 16(2): 145–150. https://doi.org/10.1080/10412905.2004.9698679

Elafify M, Bakry A M, Tian H & Huang J (2025). Phytochemicals as natural antimicrobials: a promising strategy for food safety and foodborne pathogens control. *Journal of Food Safety* 45(1): e70013. https://doi.org/10.1111/jfs.70013

Electronic Code of Federal Regulations (2025). *Title 21 — Food and Drugs; Part 182 — Substances Generally Recognized as Safe; § 182.20 Essential oils, oleoresins (solvent-free), and natural extractives (including distillates)*. U.S. National Archives and Records Administration. Displaying title 21, updated as of August 12, 2025; Title 21 last amended August 8, 2025. Retrieved 14.08.2025, from https://www.ecfr.gov/current/title-21/part-182/section-182.20

Farrukh M, Munawar A, Nawaz Z, Hussain N, Hafeez A B & Szweda P (2025). Antibiotic resistance and preventive strategies in foodborne pathogenic bacteria: a comprehensive review. *Food Science and Biotechnology* 34: 2101-2129. https://doi.org/10.1007/s10068-024-01767-x

Hyldgaard M, Mygind T & Meyer RL (2012). Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Frontiers in microbiology* 3: 12. https://doi.org/10.3389/fmicb.2012.00012

International Organization for Standardization (1999). *ISO 14715:1999 – Oil of thyme containing thymol, Spanish type* (*Thymus zygis* (*Loefl.*) *L.*). Geneva, Switzerland: ISO.

Janda J M & Abbott S L (2010). The genus Aeromonas: taxonomy, pathogenicity, and infection. *Clinical Microbiology Reviews* 23(1): 35-73. https://doi.org/10.1128/cmr.00039-09

Joshi V K, Sharma R & Kumar V (2011). Antimicrobial activity of essential oils: A Review. *International Journal of Food and Fermentation Technology* 1(2): 161-171

Kahraman Yılmaz D & Berik N (2023). Farklı bitki ekstrakt ve esansiyel yağlarin lakerda örneklerinden izole edilen bakteriler üzerine antibakteriyel etkilerinin araştirilmasi. *Çanakkale Onsekiz Mart University Journal of Marine Sciences and Fisheries* 6(1): 44-55. https://doi.org/10.46384/jmsf.1291400

Kahraman Yılmaz D & Berik N (2024). Phenotypic and genotypic antibiotic resistance of *Staphylococcus warneri* and *Staphylococcus pasteuri* isolated from stuffed mussels. *Aquatic Sciences and Engineering* 39(3): 172-178. https://doi.org/10.26650/ASE20241397385

Kahraman Yılmaz D & Berik N (2025). Phenotypic and Genotypic Antibiotic Resistance of Bacteria Isolated from Ready-to-eat Salted Seafood. *Aquatic Sciences and Engineering* 40(1): 9-17. https://doi.org/10.26650/ASE20241589821

Kerekes E B, Deák É, Takó M, Tserennadmid R, Petkovits T, Vágvölgyi C & Krisch J (2013). Anti-biofilm forming and anti-quorum sensing activity of selected essential oils and their main components on food-related micro-organisms. *Journal of Applied Microbiology* 115(4): 933-942.

Marchese A, Orhan I E, Daglia M, Barbieri R, Di Lorenzo A, Nabavi SF, Gortzi O, Izadi M & Nabavi S M (2016). Antibacterial and antifungal activities of thymol: A brief review of the literature. *Food Chemistry* 210: 402–414. https://doi.org/10.1016/j.foodchem.2016.04.111

Morales R (1986). Taxonomía del género *Thymus* L. Excluída la Sect. Serpyllum (Miller) Benthan en la Península Ibérica. Ruiza 3. C.S.I.C., Madrid.

Olaimat A N, Ababneh A M, Al-Holy M, Al-Nabulsi A, Osaili T, Abughoush M, Ayyash M & Holley R A (2024). A review of bacterial biofilm components and formation, detection methods, and their prevention and control on food contact surfaces. *Microbiology Research* 15(4): 1973–1992. https://doi.org/10.3390/microbiolres15040132

Othman L, Sleiman A & Abdel-Massih R M (2019). Antimicrobial activity of polyphenols and alkaloids in middle eastern plants. *Frontiers in Microbiology* 10: 911. https://doi.org/10.3389/fmicb.2019.00911

Pei R S, Zhou F, Ji B P & Xu J (2009). Evaluation of combined antibacterial effects of eugenol, cinnamaldehyde, thymol, and carvacrol against *E. coli* with an improved method. *Journal of Food Science* 74(7): M379–M383. https://doi.org/10.1111/j.1750-3841.2009.01287.x

Pina-Vaz C, Gonçalves Rodrigues A, Pinto E, Costa-de-Oliveira S, Tavares C, Salgueiro L, Cavaleiro C, Gonçalves M J & Martinez-de-Oliveira J (2004). Antifungal activity of Thymus oils and their major compounds. *Journal of the European Academy of Dermatology and Venereology* 18(1): 73-78. https://doi.org/10.1111/j.1468-3083.2004.00886.x

Radi F Z, Bouhrim M, Mechchate H, Al-zahrani M, Qurtam A A, Aleissa A M, Drioiche A, Handaq N & Zair T (2022). Phytochemical Analysis, Antimicrobial and Antioxidant Properties of *Thymus zygis* L. and *Thymus willdenowii* Boiss. Essential Oils. *Plants* 11(1): 15. https://doi.org/10.3390/plants11010015

Rota M C, Herrera A, Martínez R M, Sotomayor J A & Jordán M J (2008). Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. *Food Control* 19(7): 681-687. https://doi.org/10.1016/j.foodcont.2007.07.007

Sáez F (1995). Essential oil variability of *Thymus zygis* growing wild in southeastern Spain. *Phytochemistry* 40(3): 819-825

Šojić B, Milošević S, Savanović D, Zeković Z, Tomović V & Pavlić B (2023). Isolation, bioactive potential, and application of essential oils and terpenoid-rich extracts as effective antioxidant and antimicrobial agents in meat and meat products. *Molecules* 28(5): 2293. https://doi.org/10.3390/molecules28052293

Tomás J M (2012). The main Aeromonas pathogenic factors. *International Scholarly Research Notices* 2012(1): 256261. https://doi.org/10.5402/2012/256261

Touati A, Mairi A, Ibrahim N A & Idres T (2025). Essential Oils for Biofilm Control: Mechanisms, Synergies, and Translational Challenges in the Era of Antimicrobial Resistance. *Antibiotics* 14(5): 503. https://doi.org/10.3390/antibiotics14050503

Vukić M D, Čmiková N, Ben Hsouna A, Ben Saad R, Garzoli S, Schwarzová M, Vuković N L, Obradović A D, Matić M M, Waszkiewicz-Robak B, Kluz M I & Kačániová M (2023). *Thymus zygis*, valuable antimicrobial (*In vitro* and *In situ*) and antibiofilm agent with potential antiproliferative effects. *Plants* 12(23): 3920. https://doi.org/10.3390/plants12233920

Wu Y, Jiang L, Ran W, Zhong K, Zhao Y & Gao H (2024). Antimicrobial activities of natural flavonoids against foodborne pathogens and their application in food industry. *Food Chemistry* 460(1): 140476. https://doi.org/10.1016/j.foodchem.2024.140476

Ziegler H (2007). *Flavourings: production, composition, applications, regulations*. John Wiley & Sons

Copyright © 2026 The Author(s). This is an open-access article published by Faculty of Agriculture, Ankara University under the terms of the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium or format, provided the original work is properly cited.