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FEBRUARY 20-22, 2026
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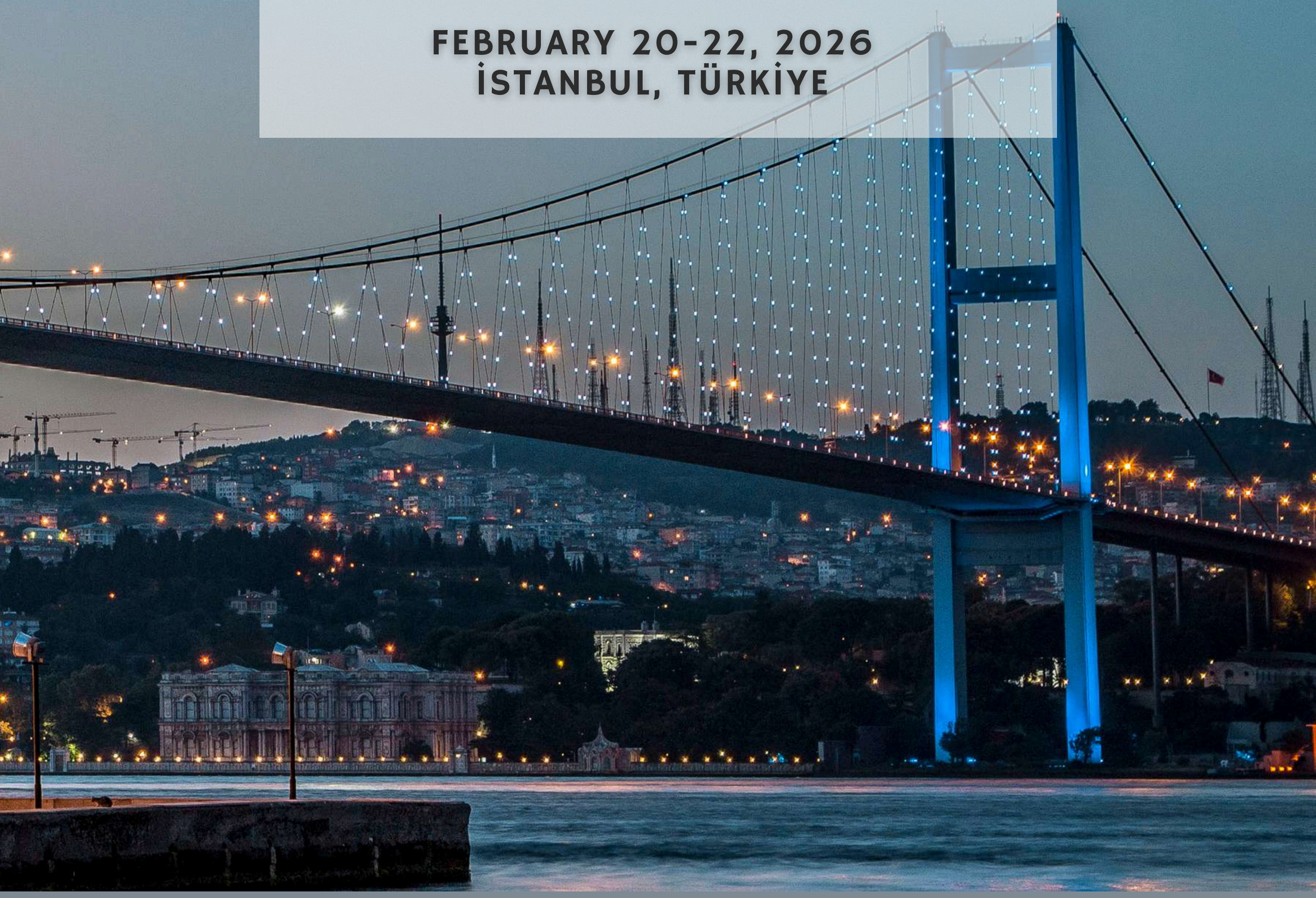

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Kurumunuz Fen Fakültesi, Analitik Kimya Anabilim Dalı'nda öğretim üyesi olarak görev yapan Prof. Dr. Habibe ÖZMEN'in 20-22 Şubat 2026 tarihinde ZOOM üzerinden çevrimiçi; İstanbul'da yüz yüze olarak gerçekleşecek ***24. Uluslararası İstanbul Fen, Mühendislik, Mimarlık ve Matematik Bilimlerinde Bilimsel Araştırmalar Kongresi***'nde açılış konuşmacısı, bilim ve organizasyon kurulu üyesi olarak yer almasını talep eder; ilgili görevlendirme yazısını tarafınızca arz ederiz.

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THYMUS ARGAEUS BOISSIER & BALANSA (LAMIACEAE): INSECTICIDAL ACTIVITY AGAINST *GALLERIA MELLONELLA* (L.) (LEPIDOPTERA:PYRALIDAE)

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ABSTRACT

Chemical and biological substances used as pesticides increase the costs of sustainable control technologies due to the toxic effects on target organisms. Therefore, the use of environmentally friendly biopesticide candidates is of great importance. In this context, the insecticidal activity of the aerial part extract of *Thymus argaeus* Boissier & Balansa (Lamiaceae) on the larval stage of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) was investigated. *G. mellonella* larvae were sterilized and exposed to different concentrations of extract (25 mg/mL, 20 mg/mL, 15 mg/mL, 10 mg/mL, 5 mg/mL, and 1 mg/mL). Mortality rates and LC_{50} and LC_{99} values were determined for the larvae using this treatment. In addition, to assess oxidative stress, the antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST), as well as the activity of acetylcholinesterase (AChE) and malondialdehyde (MDA) levels were determined. In the evaluation of *G. mellonella* larvae, it was determined that mortality rates increased significantly as the extract concentration increased, and the results of the study revealed that the extract exhibited a dose-dependent toxic effect. Biochemical findings showed a decrease in antioxidant enzyme activities and acetylcholinesterase (AChE) activity, while malondialdehyde (MDA) levels increased. When the obtained data are evaluated together, it is seen that the water extract of *Thymus argaeus* causes oxidative stress in *G. mellonella* larvae, and these findings indicate that this effect has significant potential in terms of insect control.

Keywords: Insecticidal activity**,** antioxidant enzymes, *Thymus*, biopesticide

INTRODUCTION

The larger wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), is regarded as one of the most detrimental pests in honeybee colonies and stored combs globally (Ellis et al. 2013). The economic significance of wax moths has led to comprehensive study on their life cycle, biology, behavioral

traits, ecology, molecular biology, physiology, and management measures. (Ellis et al. 2013; Kwadha et al. (2017).

Despite being considerable pests in apiculture, existing literature indicates that wax moths are extensively utilized as model organisms, especially in the fields of insect physiology, genomics, proteomics, and other molecular studies (Mikulak et al., 2018; Wojda et al., 2020; Vogel et al., 2011). The use of pesticides worldwide presents huge environmental and economic challenges due to their heavy reliance on these chemicals and how they are being implemented on an extensive level across the globe. Some of the negative effects of synthetic pesticides, especially when concerning nontarget species, their longevity in the environment, and the limited ability for them to be broken down in a biodegradability manner present serious problems for the protection of the environment. Therefore, we must develop sustainable, environmentally friendly, and economically viable alternatives to control pests (Ngegba et al., 2022). As awareness grows regarding environmental stewardship, the demand for pesticide alternatives made from plants has substantially grown. Pesticides from plants are made from plant products that kill many insects that damage crops; they can break down rapidly in the environment and can be found at a reasonable cost; they are also readily available and pose little risk to beneficial organisms such as humans (Ngegba et al., 2022).

The unique modes of action of many different plant species against pests and diseases are attributed to the phytochemical composition of these plants, making plant-based pesticides a versatile & sustainable alternative to synthetic pesticides (Ngegba et al., 2022). The composition and biological activity of plant-derived materials/ products depend on several plant-specific variables such as the parts of the plant used, the method of extraction, the age of the plant and the genotype of the plant (Angioni et al., 2006). Several documents and studies have indicated the potential of using plant-derived or vegetable products as treatments for a variety of harmful insects (Ayvaz et al., 2009; Bachrouch et al., 2010; Ercan et al., 2018; Elif et al., 2023). The *Thymus* genus, which belongs to the family Lamiaceae, is an aromatic and medicinal plant genus that is grown throughout the world, especially in the Mediterranean region (El Yaagoubi et al., 2021). The *Thymus* genus has over 200 species, the majority of which have adapted to survive in the Mediterranean Environment with large populations found in both natural and agricultural areas (El Yaagoubi et al., 2021). The essential oils from *Thymus* species are rich in phytochemical compounds such as flavonoids, phenylpropanoids, and terpenes that show a variety of biological properties including antibacterial, antifungal, antioxidant and insecticide. (El Yaagoubi et al., 2021).

The genus has been recognized as "used for food seasoning as well as for use in traditional medicine; today, they have become a popular topic in recent studies on pesticides and pharmaceutical products, due to the biological activities associated with their essential oils" (El Yaagoubi et al., 2021; Etri and Plushar, 2024). In Turkey, thymus species are abundant but have varying morphological

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characteristics as well as differences in phytochemical and ecological characteristics (Yıldız et al., 2004; Kutukalan, 2019).

The present study examined both the chemical composition of *Thymus argaeus* extract and its insecticidal effects on *Galleria mellonella* (honeybee moth). Oxidative stress is a known biochemical indicator representing a state of oxidative imbalance, which is caused by chemical pressure (i.e., excessive generation of reactive oxygen species or ROS) and the inability of cells to complete a sufficient number of antioxidants. The concentration of malondialdehyde (MDA) and the activity of free radical scavenging enzymes (e.g., superoxide dismutase [SOD], catalase [CAT], glutathione-S-transferase [GST] and glutathione peroxidase [GPx]) are some of the most frequently used indicators of oxidative damage and provide insight into how changes in the activity of these systems affect the oxidative stress experienced by the cell (Baş & Kalender, 2011).

Due to synthetic pesticide being persistent to the environment and having adverse effects on non-target forms of life; there is a need for developing alternate/safer methods of control. Therefore, the study conducted an evaluation of the potential for using *Thymus argaeus* extract as an insecticide against *Galleria mellonella.* Although there have been many studies reported in the literature regarding the insecticidal properties of several species of *Thymus*, not many studies demonstrate how *Thymus argaeus* Aqueous Extract (TAAE) would affect *Galleria mellonella.* The present study is intended to enhance the limited knowledge regarding whether the plant's Aqueous Extract could serve as a potential biopesticide candidate.

MATERIALS AND METHODS

The *Thymus argaeus* used in the study was collected from Mount Erciyes in the Kayseri region of Turkiye. The chemicals necessary for the research were purchased from the Sigma-Aldrich company. After the above-ground parts of the collected plant samples were cleaned, they were dried according to Davis (1972)'s herbarium drying procedures.

The extraction process was carried out in two stages, resulting in an aqueous extract. In the first stage, a 4 g dried plant sample was extracted with 40 mL of distilled water in the temperature range of 80–105 °C. The plant residue remaining after the first extraction was subjected to a second extraction using 60 mL of distilled water at 100–130 °C. The obtained aqueous extracts were filtered after being cooled to 25 °C, and the two fractions were combined (Mai et al., 1990).

Insect farming, extract application, and tissue collection

Different concentrations of extract (25 mg/mL, 20 mg/mL, 15 mg/mL, 10 mg/mL, 5 mg/mL, and 1 mg/mL) were applied to *Galleria mellonella* larvae. After the sterilization of the larvae, the extract was injected into the left hind leg using a microsyringe (Alvandial et al., 2016). 24 hours after the application, the larvae were cooled, and then the samples were divided into small pieces and placed into Eppendorf tubes. A pH 7.4 homogenization buffer was added to the samples (Büyükgüzel &

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Kalender, 2009), and the samples were homogenized and centrifuged. The obtained supernatants were used in biochemical analyzes.

In the supernatants, CAT, SOD, GPx, GST, and AChE enzyme activities, as well as MDA levels, were determined using a UV–VIS spectrophotometer. MDA level was determined according to the Ohkawa et al.'s (1979) method; SOD activity was determined according to the Marklund and Marklund's (1974) method; CAT activity was determined according to the Aebi's (1984) method; GPx activity was determined according to the Paglia and Valentine's (1987) method; GST activity was determined at 340 nm according to the Habig et al.'s (1974) method; and AChE activity was determined at 412 nm according to the Ellman et al.'s (1961) method.

Data Analysis

The obtained experimental data were statistically evaluated. The application means were subjected to ANOVA, Tukey's multiple comparison test, and Probit analyzes to determine inter-group differences and calculate LC_{50} and LC_{99} values. All statistical analyzes were conducted using the SPSS software package (SPSS Statistics Program, Version 20.0, SPSS Inc., Chicago, IL, USA). The data are presented as mean ± standard deviation, and a $p < 0.05$ value is considered statistically significant (Apaydın et al., 2015; Abbott, 1925).

RESULTS

Mortality data, lethal dose values, and biochemical parameters related to oxidative stress and neurotoxicity were measured after *Galleria mellonella* larvae exposure to *Thymus argaeus* water extract for 24 hours. It was observed that larval mortality increased with increasing extract concentration.With respect to the treated groups at concentrations of 1 and 5 mg/mL, the number of larvae that died in the treated groups was not significantly different from those in the control group. However, at concentrations of 10 mg/mL and greater, treated larvae experienced much higher levels of mortality, approximately 96%, at 25 mg/mL. The dose-mortality relationship of the extract used is clearly shown in the given figure (Figure 1).

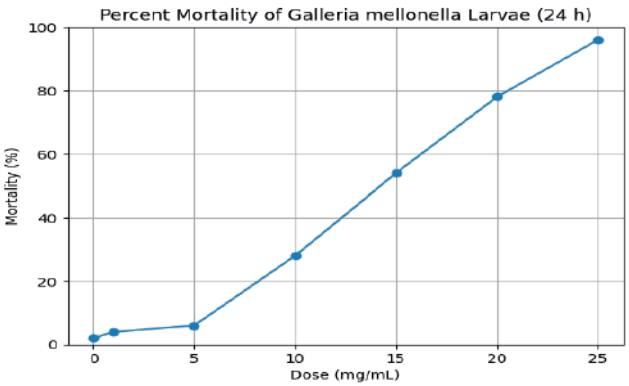

Figure 1: % mortality values of *Galleria mellonella* larvae

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Probit analysis has revealed the dose-dependent toxic effect of the extract. The LC_{50} and LC_{99} values were determined as 12.4 mg/mL (95% CI: 10.8–14.1) and 26.9 mg/mL (95% CI: 23.5–31.2), respectively. The probit regression model (Table 1) showed good fit to the mortality data, supported by a non-significant chi-square value ($p > 0.05$) and a heterogeneity factor close to one. The slope value of 2.31 ± 0.28 indicates that the dose-response relationship is consistent and reliable (Table 2).

Table 1. Probit analysis parameters for the insecticidal activity of *Thymus argaeus* water extract against *Galleria mellonella* larvae (24 h)

Parameter	**Value (mg/mL)**	**95% Confidence Interval**
LC_{10}	6.2	4.9 – 7.6
LC_{20}	8.4	7.1 – 9.8
LC_{30}	10.2	8.9 – 11.7
LC_{50}	**12.4**	**10.8 – 14.1**
LC_{70}	15.8	13.9 – 18.0
LC_{90}	22.3	19.8 – 25.9
LC_{99}	**26.9**	**23.5 – 31.2**

Table 2. Probit regression statistics

Parameter	**Value**
Slope ± SE	2.31 ± 0.28
Intercept	−5.74
Chi-square (χ^2)	3.12
Degrees of freedom (df)	4
p-value	0.54
Heterogeneity factor	1.08
Correlation coefficient (r)	0.97

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Biochemical analyzes have shown that the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST), as well as acetylcholinesterase (AChE) activity, did not show a significant change compared to the control group at concentrations of 1 and 5 mg/mL ($p > 0.05$). In contrast, a significant and gradual decrease in the activities of these enzymes was observed in the concentration range of 10–25 mg/mL ($p < 0.05$). The most significant decreases were observed in the highest dose groups, and these findings indicate that the extract exerts a strong inhibitory effect on the antioxidant defense system and AChE activity.

Unlike enzyme activities, malondialdehyde (MDA) levels showed a significant dose-dependent increase. In the 1 and 5 mg/mL application groups, MDA levels were found to be similar to the control group, while exposure to higher concentrations caused a significant increase in lipid peroxidation. The highest MDA levels were observed in the 25 mg/mL group ($p < 0.05$) (Table 3).

Table 3. Effects of *Thymus argaeus* water extract on antioxidant enzymes, AChE activity and MDA levels in *Galleria mellonella* larvae (24 h)

Group	SOD (U/mg protein)	CAT (µmol/mg protein)	GPx (U/mg protein)	GST (nmol/mg protein)	AChE (mU/mg protein)	MDA (nmol/mg protein)
Control	22.0 ± 1.8	48.4 ± 3.2	9.8 ± 0.5	62.2 ± 4.2	18.3 ± 1.3	2.1 ± 0.2
1 mg/mL	21.8 ± 2.0	47.9 ± 3.1	9.6 ± 0.6	61.8 ± 4.0	18.1 ± 1.2	2.2 ± 0.2
5 mg/mL	21.5 ± 1.8	47.2 ± 3.0	9.4 ± 0.6	61.1 ± 3.9	17.9 ± 1.2	2.3 ± 0.2
10 mg/mL	17.3 ± 1.5*	39.6 ± 2.8*	7.6 ± 0.5*	52.4 ± 3.6*	14.5 ± 1.0*	3.1 ± 0.3*
15 mg/mL	14.2 ± 1.3*	33.4 ± 2.4*	6.0 ± 0.4*	45.1 ± 3.2*	12.3 ± 0.9*	3.8 ± 0.3*
20 mg/mL	11.5 ± 1.1*	27.1 ± 2.0*	4.8 ± 0.2*	37.6 ± 2.8*	10.1 ± 0.8*	4.8 ± 0.4*
25 mg/mL	9.3 ± 0.8*	21.8 ± 1.7*	3.8 ± 0.3*	30.9 ± 2.4*	8.1 ± 0.7*	5.6 ± 0.4*

** Statistically significant difference compared to the control group ($p < 0.05$). Values are expressed as mean ± standard deviation (SD).*

Overall, the increase in observed mortality rates and the determined lethal concentration values have shown consistency with the biochemical changes detected in antioxidant enzyme activities, AChE activity, and MDA levels. These findings indicate that the water extract of *Thymus argaeus* induces concentration-dependent toxicity in *Galleria mellonella* larvae and that this effect is likely manifested thru the induction of oxidative stress and the impairment of neurophysiological functions.

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DISCUSSION

Recently conducted research evaluated the insecticidal properties of *Thymus argaeus* aqueous extract on *Galleria mellonella* larvae using both mortality and biochemistry as dependent variables. Results demonstrate a dose-dependent relationship between concentration of extract and toxicity; toxicity increases as concentration of extract increases in a predictable manner. Mortality increased dramatically beginning at concentrations of 10mg/mL or greater, and an LC_{50} value of 12.4mg/mL indicates that *T. argaeus* has high potential for insecticidal activity. The findings of this study correlate with several studies in the literature that suggest that *Galleria mellonella* is a sensitive model organism for use in toxicity and stress biology studies (Dubovskiy et al., 2008; BüyükGüzel & Kalender, 2009).

The consistent relationship between increasing larval death with increasing concentration and the moderate LC_{50} value suggest that this extract has a systematic and significant biological toxicity. Other researchers have found that mortality from exposure to toxic agents by *Galleria mellonella* tends to follow a classic dose-response relationship, with increased mortality associated with changes to oxidative stress parameters (Dubovskiy et al., 2008; Büyükgüzel and Kalender, 2009; Tunçsoy, 2021). These authors also found that there is a strong correlation between increased oxidative stress due to increased toxicity for *Galleria mellonella* and the suppression of antioxidant enzyme levels (SOD, CAT, GPx and GST) and levels of MDA (Dubovskiy et al., 2008). The simultaneous suppression of the antioxidant defense system with increasing larval mortality in this study indicates that the toxic effect of the extract extends beyond lethality and includes a physiological mechanism due to a disruption of cellular redox homeostasis. Additional studies using nanoparticles and other toxic chemical compounds have also shown parallel increases in oxidative stress parameters and mortality rates in *Galleria mellonella* (Tunçsoy, 2021; Büyükgüzel et al., 2010).

The significant decrease observed in antioxidant enzyme activities (SOD, CAT, GPx, GST) along with the increase in MDA levels indicates that the extract creates strong oxidative stress in larval tissues. In *Galleria mellonella*, an increase in lipid peroxidation and an imbalance of antioxidant enzymes have been similarly reported under bacterial infection, chemical agents, or environmental stress conditions (Dubovskiy et al., 2008; Büyükgüzel et al., 2010). Antioxidant enzymes form the first line of defense in the detoxification of reactive oxygen species, and the suppression of this system triggers lipid peroxidation in the cell membrane (Halliwell & Gutteridge, 2015). An increase in MDA levels is an indicator of oxidative degradation and is considered a reliable biomarker of oxidative damage (Del Rio et al., 2005). Similar increases in MDA levels have been reported in the *Galleria mellonella* model following exposure to various toxic agents (Büyükgüzel & Kalender, 2009). These parallel supports that the aqueous extract of *Thymus argaeus* causes cellular damage by disrupting redox homeostasis. The decrease in GST activity indicates a reduced capacity of the larvae to neutralize toxic compounds. Glutathione S-transferase (GST) enzymes bind reactive oxidative byproducts and lipid peroxidation derivatives formed in the cell with glutathione, making them more water-soluble and less harmful. The

weakening of this conjugation mechanism leads to the accumulation of toxic intermediates within the cell and an increase in oxidative damage (Enayati et al., 2005; Büyükgüzel et al., 2010).

A marked decline in AChE activity shows that the extract is acting as a source of metabolic pressure and neurotoxic damage. Inhibition of AChE can result in excess acetylcholine in the synaptic cleft (space between two neurons), causing excessive stimulation of the nervous system and impaired coordination of the muscles in the body (Colovic et al., 2013). In addition, plant-derived volatile oils have been shown to cause disruption of neurophysiological balance due to activation of octopamine receptors, which are specific to the insect's neurotransmitter system; thus, demonstrating that other forms of neurotoxic activity also exist within the essential oil group (Kostyukovsky et al., 2002). Essential oils from various species have also been found to have insecticidal properties against Lepidoptera and Coleoptera, and evidence exists that the insecticidal effects of essential oils are due to their actions upon the nervous system (Polatoğlu et al., 2016; Sümer Ercan et al., 2013).

Increased mortality, decreased antioxidant enzyme activity, increased MDA, and decreased activity of AChE activity indicate that the extract has a multi-targeted mode of action. Such a mode of action associated with botanical insecticides indicates a reduced potential for the development of resistance compared to a single-target mode of action associated with synthetic insecticides (Regnault-Roger et al., 2012; Pavela, 2015).

According to the present study, when injected with the aqueous extract of *Thymus argaeus*, the *Galleria mellonella* larvae will die due to a concentration dependent toxicity associated with increased oxidative stress and cholinergic disruption; thus the lactate dehydrogenase (LDH) assay, glutathione oxidase (GDH) assay, antioxidant enzyme assays and malondialdehyde (MDA) activity will all show elevated values in relation to the increasing concentration of *T. argaeus.* The increasing elevation of the LDH activity level supports the findings of the increased oxidation, as shown by the LEDG and MDA activities.

The information above about the *Galleria mellonella* is all cell based, making it a perfect model organism to study the mechanisms behind the phytotoxicity of the extracts studied. Further studies are needed to examine the production of reactive oxygen species (ROS), changes in gene expression, and intracellular signaling pathways in order to better understand the phytotoxic effects of *T. argaeus.*

CONCLUSION

This study unequivocally illustrates that the aqueous extract of *Thymus argaeus* has a potent and concentration-dependent insecticidal activity on *Galleria mellonella* larvae. The rising mortality rates, the assessment of the LC_{50} value, and concurrent biochemical disruptions suggest that the extract possesses both lethal effects and a multi-faceted toxicity profile by impairing cellular redox equilibrium and influencing cholinergic nerve transmission. The suppression of the antioxidant defense system and the increase in lipid peroxidation highlight the significant impact of oxidative

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stress, while AChE inhibition indicates a disturbance at the neurophysiological level. The comprehensive impact profile indicates that *Thymus argaeus* may serve as a viable choice for the formulation of natural pesticides. The identification of active components and the execution of confirmatory investigations at the molecular level are essential for converting this promise into practical implementation.

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