

## The effect of guaiazulene on the survival of the fall webworm *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) larvae

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### Abstract

Since plants are sessile organisms, they have evolved various mechanisms to combat herbivores attacking them. One of these adaptations is the production of terpenes, a form of secondary metabolite. In our study, we determined the effect of guaiazulene, a terpene type, on the survival of *Hyphantria cunea* larvae. For this reason, the 1st generation larvae were collected from Bafra District of Samsun Province, Turkey in 2020 and were let to feed on the control diet until the pupal stage. Individuals originating from the 1st generation adults fed on the control diet in the first two larval stages and fed on the artificial diets containing different concentrations of guaiazulene in the third larval stage used in our study. As a result of our study, we found that the mortality rate of the larvae exposed to increasing concentration of guaiazulene increased. Based on the results of this study, we suggest that guaiazulene is toxic to the larvae and can be used in pest control as an alternative insecticide.

**Keywords:** survival, guaiazulene, *Hyphantria Cunea*, terpene, toxicity

### Introduction

Plants are sessile organisms that have evolved diverse mechanisms to adapt to complex and changing environments. The production of specialized metabolites is an essential feature of plant adaptation. Terpenes are the largest class of specialized metabolites with more than 80,000 structures reported to date. These metabolites consist of five-carbon isoprene units and can be subdivided into monoterpenes (C<sub>10</sub>), sesquiterpenes (C<sub>15</sub>), diterpenes (C<sub>20</sub>), sesterterpenes (C<sub>25</sub>), triterpenes (C<sub>30</sub>), and higher terpenes (>C<sub>30</sub>) such as carotenoids (Huang and Osbourn, 2019) [20]. The role of terpenes against herbivores and pathogens has been demonstrated in several studies (Björkman and Larsson, 1991; Hilker *et al.*, 2002; Nykanen and Koricheva, 2004; Beyaert *et al.*, 2012) [5, 18, 33, 4]. Among these metabolites, the well-known roles of monoterpenes and sesquiterpenes in plants are their activities as constitutive or induced defensive compounds with direct or indirect effects against herbivores or pathogens (Kessler and Baldwin, 2001; Rasmann *et al.*, 2005; Chiu *et al.*, 2017) [22, 37, 9]. These metabolites with low molecular weight are volatile in ambient conditions due to their high vapor pressure (Dudareva *et al.*, 2004) [12]. They attract pollinator insects or decrease pathogen infections due to these properties (Huang *et al.*, 2012; Raguso, 2016) [21, 36].

Guaiazulene (1,4-dimethyl-7-isopropylazulene) is a bicyclic sesquiterpene derived from the different plants, the guaiac wood oil, *Callis intratropica* blue, and *Matricaria chamomilla* L. (O'Neal *et al.*, 2006; Fiori *et al.*, 2011) [34, 14] and has a fascinating blue color since the late medieval period (Amir *et al.*, 2011) [2]. It has attracted much attention due to its beneficial biological activities (Fiori *et al.*, 2011) [14]. Studies have shown that this substance has relaxing properties as well as antioxidant, antifungal, antimicrobial, anti-inflammatory, antispasmodic, and anti-ulcer activities (Yanagisawa *et al.*, 1990; Andersen, 1999; Tanaka *et al.*, 2000; Guarrera *et al.*, 2001; Fiori *et al.*, 2008; Sleha *et al.*,

2014) [44, 3, 40, 17, 13, 38].

The fall webworm *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) is an insect native to North America. This insect feeds on the leaves of several plants, spreads rapidly, and causes significant ecological and economic losses (Ge *et al.*, 2019) [16]. It is known that this insect feeds on around 600 species of plants, including fruit and forest trees, shrubs, ornamental plants, and some herbaceous plants (Lu *et al.*, 2017) [27]. It also causes a loss of many economically important crops in Turkey.

It is recognized that the natural products derived from the plants can be new sources of insect pest control. In this case, a group of potentially valuable allelochemical is terpenoids (García *et al.*, 2003) [15]. The functions of these metabolites have been studied in various insects (Ortego *et al.*, 1999; García *et al.*, 2003; Beyaert *et al.*, 2012) [35, 15, 4]. Our aim in this study is to demonstrate that guaiazulene can be used as an alternative insecticide in the control of *H. cunea* larvae.

### Materials and Methods

#### Obtaining larvae and experimental setups

The first generation of *H. cunea* larvae was collected from Bafra District of Samsun Province, Turkey in 2020. The larvae brought to the laboratory were kept at 25°C (16 h light/8 h dark) and were let to feed on the diet (control diet) developed by Yamamoto (1969) [43] until they reached the pupal stage. The adults that emerged from the pupae mated and laid eggs. The hatched insects fed on the control diet at the 1st and 2nd larval stages. The control diet was modified for our study in which the 3rd larval stage was used. Guaiazulene used in the diet was provided from Sigma-Aldrich Chemistry. Dimethyl sulfoxide (DMSO) was used as the solvent for the dissolution of guaiazulene at a ratio of 1% (Bohbot and Dickens, 2010) [6]. Guaiazulene dissolved in DMSO was added to the control diet at concentrations of 25, 50, 100, and 200 mg/L. These concentrations were determined based on the study of Togar *et al.* (2015) [42]. A

total of the five different diets were included in our study, one of which was a control diet (Table 1).

In this study, the thirty larvae were placed in each group to determine survival rates, and each experiment was replicated three times. A total of 450 larvae, the ninety larvae in each group, were used.

### Statistical analysis

Two independent samples t-test was used to determine the survival rate depending on the diet content. The Kaplan-Meier test was used for statistical significance of differences in the survival of *H. cunea* larvae fed on the diets containing various concentrations of guaiazulene. The survival rates of the larvae fed with various concentrations of guaiazulene compared to the control group were compared with the Log-Rank test. The lethal dose (LC<sub>50</sub> and LC<sub>90</sub>) was calculated by the probit analysis. SPSS 21.0 software was used for these tests.

### Results and Discussion

Investigation of the insecticidal properties of plant-derived bioactive metabolites has recently gained significance. Many of the plant-derived metabolites may be used as harmful substances and they have detrimental impacts on insect pests (Leicach and Chludil, 2014; Mouden *et al.*, 2017; Michael, 2018) [26, 30, 28]. Dietary stress from toxic exposure may dramatically affect the herbivorous insects. Terpenes, the largest secondary metabolites class, protect numerous species of plants by showing the harmful effects against pests and pathogens (Dicke and Baldwin, 2010) [11]. The role of terpenes against herbivores and pathogens has been demonstrated in several studies (Hodar and Zamora, 2004; Mumm and Hilker, 2006; Moreira *et al.*, 2013; Agrawal and Weber, 2015) [19, 32, 29, 1]. The insecticidal function of terpenes is due to its effects as either antifeedants or toxins. In our study, the survival rates of *H. cunea* larvae according to the Kaplan-Meier analysis were shown in Table 2 and Table 3. The survival rate in the control group was 97.8%, so the survival rates were not calculated again with the Abbott formula. The survival rates were 4.4% for the larvae fed the diet containing 25 mg/L of guaiazulene, 4.5% for those fed the diet containing 50 mg/L of guaiazulene, 2.2% for those fed the diet containing 100 mg/L of guaiazulene, and 1.1% for those fed the diet with the highest concentration of guaiazulene (200 mg/L), respectively. This finding proved that guaiazulene had a toxic effect on *H. cunea* larvae. In our study, it was determined that the mortality rate of *H. cunea* larvae increased as the amount of guaiazulene, a form of sesquiterpene, increased. This finding coincided with the result those Kordali *et al.* (2017) [25] found that the mortality rate of *Sitophilus granarius* increased with an increase in guaiazulene. In a study by García *et al.* (2003) [15] investigating the effects of certain sesquiterpenes against *Tenebrio molitor* larvae, they found that the mortality rate increased with an increase in tassaric acid concentration. In other study in which Conti *et al.* (2011) [10] applied an essential oil containing various terpenes from two different plant species to *Sitophilus granarius* adults, it was observed that the mortality rate of this insects increased with the increasing concentration. Srivastava *et al.* (1990) [39] found that a sesquiterpene was toxic to *Spodoptera littoralis* larvae and that there was a high mortality rate for the larvae. Mullin *et al.* (1991) [31] proved that certain sesquiterpenes

had toxic effects on *Diabrotica virgifera*. Khani and Heydarian (2014) [23] determined that the mortality rate of both *Callosobruchus maculatus* and *Teucrium castaneum* increased with an increase in the concentration of sesquiterpene. In our study, the larvae fed the diet containing the highest concentration of guaiazulene (D) died faster than those fed other diets. While all of the larvae fed on D diet died on the 9th day, this resulted in the death of all the larvae in groups B and C on the 14th day (Figure 1).

Sesquiterpenes are highly variable in chemical composition and are toxic to both insects and mammals. In a study conducted, it was determined that *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, the dominant species of house dust mites, died at a low dose (6.3mg/cm<sup>2</sup>) of  $\alpha$ -cadinol after 24 hours of exposure (Chang *et al.*, 2001) [7]. In another study, it was found that caryophyllene oxide showed the high insecticidal activity (LC<sub>50</sub> = 0.18 mg/L) against *Tribolium castaneum* adults (Kim *et al.*, 2010) [24]. The LC<sub>50</sub> value of caryophyllene oxide in *Cinnamomum osmophloeum* leaf essential oil against mosquito species was 65.6  $\mu$ g/mL for *Aedes albopictus* larvae (Cheng *et al.*, 2009) [8]. In our study, while the LC<sub>50</sub> value found according to the probit analysis was 1.10<sup>-4</sup> mg/ml, the LC<sub>90</sub> value was reported as 19.10<sup>-3</sup> mg/ml (Figure 2).

According to the Log-Rank test, the survival rate of the larvae fed on the control diet was statistically different from that of the other groups. It was noted that the survival rates of the larvae fed on B and C diets were not statistically different (Table 4).

### Tables and Figures

**Table 1:** Diet groups and diet contents

Diet groups	Diet contents
Control	Control diet
A	25 mg/L guaiazulene
B	50 mg/L guaiazulene
C	100 mg/L guaiazulene
D	200 mg/L guaiazulene

**Table 2:** Survival rates of *Hyphantria cunea* larvae fed on diets with various guaiazulene concentrations according to the Kaplan-Meier analysis

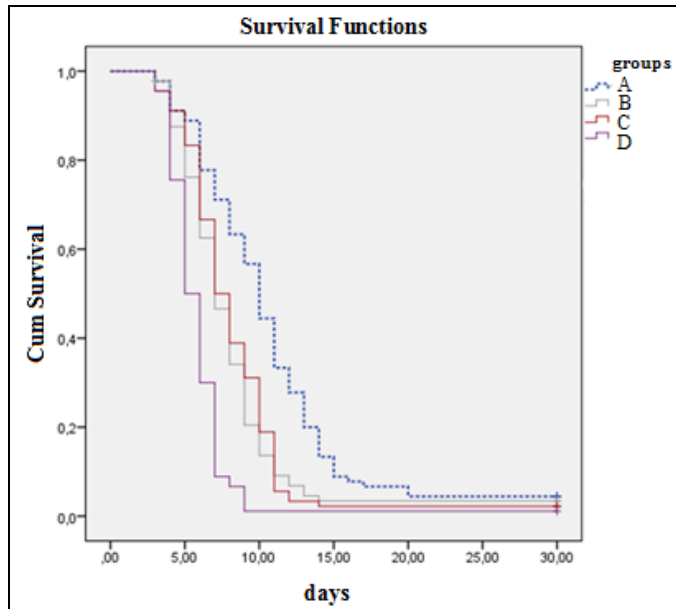
Groups	Total N	N of Events	Censored	
Control	90	2	88	97.8 %
A	90	86	4	4.4%
B	90	86	4	4.5%
C	90	88	2	2.2%
D	90	89	1	1.1%
Overall	359	348	11	3.1%

**Table 3:** The Kaplan-Meier analysis results of *Hyphantria cunea* larvae fed on diets with various guaiazulene concentrations

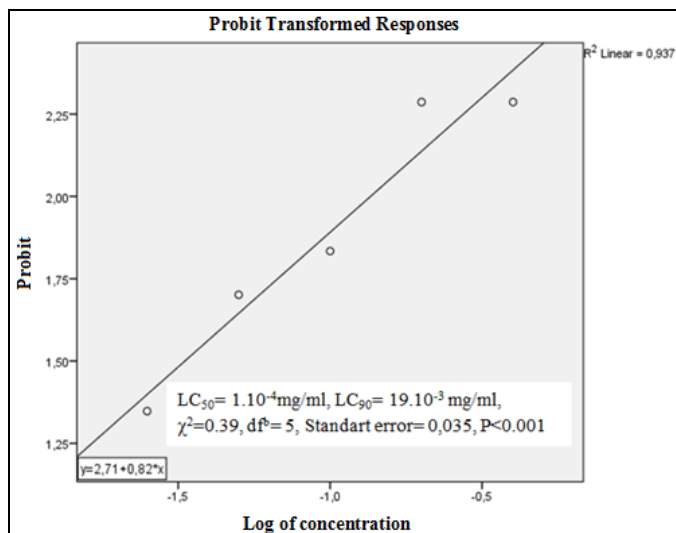
Groups	Median			
	Estimate	Std. error	95% Confidence Interval	
			Lower Bound	Upper Bound
A	10.000	.429	9.160	10.840
B	7.000	.374	6.266	7.734
C	7.000	.379	6.256	7.744
D	5.000	.231	4.546	5.454
Overall	7.000	.174	6.659	7.341

**Table 4:** Comparison of survival rates of *Hyphantria cunea* larvae fed on the diets with various guaiiazulene concentrations by the Log-Rank test

	Groups	A		B		C		D	
		$\chi^2$	Sig.	$\chi^2$	Sig.	$\chi^2$	Sig.	$\chi^2$	Sig.
Log-Rank (Mantel-Cox)	A			17.680	.000	17.236	.000	73.083	.000
	B	17.680	.000			.417	.519	28.744	.000
	C	17.236	.000	.417	.519			40.007	.000
	D	73.083	.000	28.744	.000	40.007	.000		



**Fig 1:** Survival of *Hyphantria cunea* larvae fed on the diets with various guaiiazulene concentrations according to the Kaplan-Meier analysis



**Fig 2:** Median lethal concentrations (LC<sub>50</sub>) of *Hyphantria cunea* third instar larvae post-treatment with guaiiazulene using the probit analysis

**Conclusion**

Secondary metabolites obtained from plants are the essential sources of biopesticides. Since these chemical metabolites degrade rapidly and lack persistence in the ecosystem (Theis and Lerda, 2003; Michael, 2018) [41, 28], they can be used safely. Moreover, understanding the response of herbivorous insects to plant allelochemicals provides a basis for the development and application of new botanical pesticides for pest control. In this study, the effects of

guaiazulene on the survival of the third instar of the *H. cunea* were shown. With the increasing amount of guaiiazulene, an increase in the mortality rate of the larvae was recorded. As a result of our study, it was determined that guaiiazulene had toxic properties and could potentially be used to control *H. cunea* larvae.

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