



THE EFFECT OF THYMOL ON THE DEVELOPMENT OF *DROSOPHILA MELANOGASTER*

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ABSTRACT. In this article, the effect of the plant-derived monoterpene thymol on the survivorship and development time of *Drosophila melanogaster* was investigated. *D. melanogaster* is a model organism that has been studied in many areas. The larvae of *D. melanogaster* (newly hatched) were grown with artificial diets containing different concentrations of thymol (10 mg/L, 100 mg/L, 500 mg/L, and 1000 mg/L) until the adult stage, and then the survivorship and development of the insect were investigated. The experiments were performed independently in four replications at 25±2 °C and 60-70% relative humidity and 12:12 light: dark conditions. As the thymol concentration increased, especially at high concentrations, the survival rate of the larvae, pupae, and adults of the insect decreased, but the development period was prolonged. In this study, it was determined that thymol had a negative effect on the development of *D. melanogaster*.

Keywords: *Thymol, Drosophila melanogaster, development, survival.*

INTRODUCTION

Drosophila melanogaster is in the family Drosophilidae of the order Diptera. It is a model organism that is frequently used in nutrition studies because it is similar to mammalian species. The body structure of this insect is simpler than that of mammals, but it includes organ systems with equivalent functions to the mammalian heart, lung, kidney, liver, and gonads. In particular, intestinal tissue shows characteristics similar to those of mammals [1-3]. In addition, it is physiologically, biochemically, and genetically similar to mosquitoes and flies that have medical importance. It is also preferred in nutrition studies because it is easy to maintain, lays many eggs, and reproduces quickly [4].

Long-term use of pesticides can cause negative effects on natural enemies of pests, especially on human and environmental health [5]. For this reason, there is an increase in the use of organic substances in agricultural control, especially plant-based chemicals that have little or no harmful effect on the environment or non-target organisms. As a result, essential oils and their main component, monoterpenes, are bioactive compounds used in the fight against insects. Insects can be affected by essential oils through feeding, inhalation, or skin contact. Essential oils indicate their insecticidal effects in the form of

reduced growth, affected molting, prolonged development, behavioral changes, midgut membrane disruption, metabolic disorders, neuromuscular toxicity, and non-specific multisite inhibitions [6]. Nutrients are simple organic molecules that undergo biochemical reactions that produce energy in animals after digestion [7]. There is a mutual relationship and interaction between the insect and the plant in order to perform their vital activities such as feeding, sheltering, and laying eggs. Nutritional studies show that nutritional compounds and plant metabolites have an effect on insects [8, 9].

Thymol (2-isopropyl 5-methylphenol), a plant secondary metabolite, is a monoterpene and is mostly found in thyme species. Monoterpenes form the fragrant property of the plant due to their low boiling point. In addition, it has an antimicrobial activity due to its phenolic structure [10]. It has long been used as an expectorant, anti-inflammatory, antiviral, antibacterial, and antiseptic agent in traditional medicine, particularly in the treatment of the upper respiratory system. Recent studies have also shown that thymol has antibiofilm, antifungal, antiviral, and anticancer properties [11]. Thymol affects the central nervous system, flight muscles, and neuromuscular junctions of the insects. This inhibitor acts on GABA-sensitive sites *in vivo* by mimicking or facilitating the effects of the neurotransmitter [12]. It is also known that this secondary metabolite has a repellent effect on different insect species [13]. However, the effect of monoterpene thymol on the biological parameters of the model organism *Drosophila melanogaster* is not known enough. In this study, the effects of the secondary metabolite thymol on the survivorship and development of the model organism *Drosophila melanogaster* were investigated.

MATERIALS AND METHODS

Drosophila melanogaster Culture in Laboratory Conditions

In this study, newly hatched larvae, pupae, and adults of *Drosophila melanogaster* (Diptera, Drosophilidae) were used. *D. melanogaster* culture was reared at 25 ± 2 °C and 60-70% relative humidity and 12:12 light: dark conditions. The diet developed by Roberts (1986) [14] was used in insect culture. The diet contains of 8 g of agar-agar ultrapure (Merck, Darmstadt, Germany), 20 g of sucrose (BioUltra, $\geq 99\%$, Sigma Chemical Co., St. Louis, MO), 11.78 g of dried powder yeast (Dr. Oetker Food Co., Torbalı-İzmir, Turkey), 0.8 g of ascorbic acid (BioUltra, $\geq 99\%$, Sigma), 7.72 ml of nipagine (SigmaUltra, p-hydroxybenzoic acid methyl ester, crystal), 36 g of mashed potatoes (Knorr, Unilever Co., Umraniye, İstanbul, Turkey), and 1,000 ml of distilled water. The prepared diet was transferred into glass bottles (about 2-3 cm) and waited for 30 minutes to solidify. Ten or fifteen female and male individuals were placed in these bottles. The mouths of the bottles were plugged with clean hydrophilic cotton to ensure adequate ventilation. From the eggs laid by the females on the food in the bottles, 1st stage, 2nd stage, and 3rd stage (the final larval stage) larvae, pups, and adult individuals were formed, respectively. When the larvae turned into pupae, the adults in the bottle were transferred to other bottles containing food [15]. This process was carried out once a week. Adults obtained from these pupae were used both for the continuity of *D. melanogaster* culture and for obtaining the eggs needed for thymol-related feeding experiments.

Obtaining Thymol and Insect Larvae Used in Experiments

Thymol (C₁₀H₁₄O) was purchased from Merck (108167). The concentrations of thymol (10 mg/L, 100 mg/L, 500 mg/L and 1000 mg/L) added to artificial diet were determined by examining previous studies on some harmful insect species, primarily *D. melanogaster* [15-18]. *D. melanogaster* larvae were grown with artificial diet containing thymol, and the survivorship and development from egg to adult were observed. 2-3 female and male adults were left on the artificial diet in the glass bottle. After the female adults lay their eggs in this diet, the survivor, and development time experiments were carried out from the larvae obtained from the hatched eggs.

Nutrition Experiments

D. melanogaster larvae (newly hatched) were grown with an artificial diet containing different concentrations of thymol (10 mg/L, 100 mg/L, 500 mg/L, and 1000 mg/L) until the adult stage, and the survivorship and development of the insect were investigated. Experiments were carried out in four replicates at 25±2 °C and 60-70% relative humidity and 12:12 light: dark conditions. The preparation of the diet used in the experiment, the obtaining of the eggs, and the inoculation of the hatched larvae from the eggs into the diet were all done under non-aseptic conditions.

Evaluation of Data Based on Results

The effects on survival and development time of *D. melanogaster* in artificial diets containing different concentrations of thymol were determined, as well as the percentage of individuals reaching the third larval stage, pupae, and adult stage, and the time it took to reach these stages (days). One-way “Analysis of Variance” (ANOVA) [19] was used to evaluate the data related to development time. The “LSD Test” [19] was applied to determine the significance of the difference between the means. “ χ^2 (Chi square) Test” [20] was used to evaluate the data related to survival. The significance of the means at the 0.05 probability level was examined.

RESULTS AND DISCUSSION

In the control group (without thymol), the rate of larvae reaching the third instar was 92.50±1.25. Comparing the control diet with diets including 10, 500, and 1000 mg/L thymol concentrations, the survival rate of the third instar larvae was significantly reduced. This rate is approximately 2 ($\chi^2=40.272$; df=1; P=0.0001) and 3 ($\chi^2=63.613$; df=1; P=0.0001) times lower in foods containing 500 mg/L and 1000 mg/L thymol, respectively, compared to the control group (Table 1).

Pupation rates were significantly reduced at all concentrations. Especially in the diet concentration containing the highest thymol (1000 mg/L), this rate is approximately 8 times ($\chi^2=96.160$; df=1; P=0.0001) lower than the control group (Table 1).

The rates of becoming adults decreased at all concentrations. Compared to the control group, the rate of becoming an adult decreased from 81.25±2.07 to 11.25±1.08 ($\chi^2=78.843$; df=1; P=0.0001) at the diet concentration containing 1000 mg/L thymol. According to these results, we can say that thymol reduces the rate of *D. melanogaster* reaching third instar larvae, pupation, and becoming an adult (Table 1).

In the control diet, the development time of *D. melanogaster* to the third instar larva is 3.50±0.10 days. It prolonged the development time of third-instar larvae in all

concentration diets containing thymol. This value increased from 3.50 ± 0.10 days to 6.28 ± 0.08 days ($F=20.717$; $df=4$; $P=0.0001$) in the 1000 mg/L concentration diet containing thymol, and this value was statistically significant (Table 2).

The pupal development time of *D. melanogaster* was 4.43 ± 0.04 days in the control group. At concentrations containing 10, 100, 500, and 1000 mg/L thymol, these values were 5.87 ± 0.21 ($F=28.005$; $df=4$; $P=0.008$), 5.73 ± 0.25 ($F=28.005$; $df=4$; $P=0.015$), 7.06 ± 0.42 ($F=28.005$; $df=4$; $P=0.0001$), and 9.12 ± 0.37 ($F=28.005$; $df=4$; $P=0.0001$) days, respectively. According to these results, it can be said that the development time of the pupa was significantly prolonged, especially at the last two concentrations containing 500 and 1000 mg/L thymol (Table 2).

In the control group, the adult development time was 8.74 ± 0.28 days. At concentrations containing 10, 100, 500, and 1000 mg/L thymol, these values were found to be 10.35 ± 0.10 ($F=73.106$; $df=4$; $P=0.0001$), 9.55 ± 0.06 ($F=73.106$; $df=4$; $P=0.003$), 11.52 ± 0.05 ($F=73.106$; $df=4$; $P=0.0001$), and 12.15 ± 0.08 ($F=73.106$; $df=4$; $P=0.0001$) days. For the diet containing 10, 500, and 1000 mg/L thymol, the development time of the adults was extended by approximately 2, 3, and 4 days, respectively (Table 2).

According to these data, we can say that the developmental period of 3rd-stage larvae, pupae, and adults is prolonged and the survival rates of larvae, pupae, and adults decrease.

Table 1. Effects of dietary Thymol (at the indicated proportions of diet) on survival of the *Drosophila melanogaster*

Thymol (mg/L)	3rd instar survival (%) (Mean [*] ±SE) [†]	Pupal survival (%) (Mean [*] ±SE) [†]	Adult survival (%) (Mean [*] ±SE) [†]
0.000 [§]	92.50±1.25 ^a	90.00±1.77 ^a	81.25±2.07 ^a
10	52.50±1.25 ^b	52.50±1.25 ^b	47.50±1.25 ^b
100	77.50±1.25 ^c	77.50±1.25 ^c	67.50±1.25 ^c
500	46.25±2.07 ^{bd}	45.00±1.77 ^d	25.00±3.06 ^d
1000	31.25±2.07 ^d	12.50±1.25 ^e	11.25±1.08 ^e

* The average of four replicates, 20 first instar larvae were used for each replicate.

† Values containing the same letter in the same column are not different from each other, $P < 0.05$ (LSD test).

§Control (without Thymol).

Table 2. Effects of dietary Thymol (at the indicated proportions of diet) on development of the *Drosophila melanogaster*

Thymol (mg/L)	3rd instar Development time (days) (Mean [*] ±SE) [†]	Pupae Development time (days) (Mean [*] ±SE) [†]	Adult Development time (days) (Mean [*] ±SE) [†]
0.000 [§]	3.50±0.10 ^a	4.43±0.04 ^a	8.74±0.28 ^a
10	4.68±0.29 ^b	5.87±0.21 ^b	10.35±0.10 ^b
100	4.73±0.26 ^b	5.73±0.25 ^b	9.55±0.06 ^c
500	5.15±0.10 ^b	7.06±0.42 ^c	11.52±0.05 ^d
1000	6.28±0.08 ^c	9.12±0.37 ^d	12.15±0.08 ^e

* The average of four replicates, 20 first instar larvae were used for each replicate.

† Values containing the same letter in the same column are not different from each other, $P < 0.05$ (LSD test, χ^2 test).

§Control (without Thymol).

Thymol (40.5%) is the active ingredient responsible for the repellent and insecticidal effect of the essential oil obtained from the thyme plant (*Thymus vulgaris*). Other major compounds are p-cymene (23.6%), carvacrol (3.2%), linalool (5.4%), β caryophyllene (2.6%), terpinen-4-ol (0.7%), borneol, 1,8-cineole, geraniol, and various other terpenoids, alcohols, and esters [21]. Studies have proven that thyme oil has a repellent effect for up to 3 hours against harmful insect species. It has been observed that thyme oil has a repellent effect against *Bemisia tabaci*, *Aedes aegypti*, *Sitophilus zeamais*, and *Plodia interpunctella* [22]. Biologically active compounds found in essential oils, such as 1-8 cineole, thymol, eugenol, limonene, carvacrol, pinene, and myrcene, have adverse effects on various insect species. These plant-derived bioactive substances can also be used as safe and environmentally friendly insecticides [23]. Many studies have been conducted in the literature to determine the insecticidal effect of essential oils against the Diptera order [24-29]. However, the effect of thymol, a monoterpenoid, on the biological parameters, which are the survivor rate and development time of *Drosophila melanogaster*, has not been investigated. According to the results obtained from this study, especially the diet containing the highest concentration of thymol (1000 mg/L) and all other concentrations prolonged the larval, pupal, and adult development periods of *D. melanogaster*.

It has been stated that essential oil components (α Pinene, β Pinene, $-\alpha$ Bisabolol, Carvacrol, Thujone, (+)-Limonene, and a mixture of all of them together) have a negative effect on the larvae and adults of *Bactrocera dorsalis* (Diptera), a species of agricultural pest. It has also been reported that the essential oil components selected in this study have the potential to be developed as an alternative to synthetic pesticides [30]. The insecticidal effect of essential oils obtained from the leaves of *Eucalyptus globulus* (Myrtales: Myrtaceae) against the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) was investigated [31]. In another study, it was reported that 1-8-cineole and camphene used against West Nile virus vector *Culex pipiens pallens* had larvicidal activity [29, 32]. It is thought that a similar effect will be exhibited by thymol.

Anopheles gambiae (Diptera: Culicidae) is a species that causes malaria. In a study conducted with adults of this species, it was stated that the essential oils of the plants and their combinations had a negative effect on the insect. A species belonging to the order Diptera was fed with monoterpene, and as a result, it caused a significant decrease in the rate of larvae and pupae [29]. Similar results were obtained in our study. When compared with the control diet, the rate of reaching third instar larvae and pupating of *D. melanogaster* fed with the diet containing the two highest concentrations of thymol (500 and 1000 mg/L) was reduced.

The diet containing the highest concentration of thymol decreased the adult rate of *D. melanogaster* by approximately 7.5 times compared to the control group. The reason for this result is that thymol added to the artificial diet may have decreased the nutrition intake in adults. The insecticidal effect of hydrolat obtained from *Monarda didyma* scarlet balsam against *Drosophila suzukii* was investigated. According to the obtained results, it affected nutrition intake in adults and also caused a decrease in the number of eggs in females [33]. Monoterpenoids are used as an integrated pest management tool for insect pests. Thymol was shown to have the highest toxicity of all the essential oils in the toxicity test carried out on *Galleria mellonella* adults [34, 35]. *D. melanogaster* is used as a model in the treatment of human diseases, especially Alzheimer's disease. In a nutrition study, *D. melanogaster* adults were fed essential oils obtained from *Citrus limon* and *C. reticulata* for 7 days. It was stated that no significant mortality occurred in adults fed with

essential oils. As a result, it has been reported that these essential oils can be used to treat the disease by preventing oxidative stress [36]. In our study, *D. melanogaster* was fed diets containing 10, 100, 500, and 1000 mg/L thymol from egg to adult stage and showed negative effects on biological parameters, especially at high concentrations, by slowing down the development time. The reason for this negative effect may be due to an essential oil obtained from a different kind of plant. Many essential oil components are considered safe for animals and readily degradable in the environment [35,37].

CONCLUSION

This study shows that the monoterpene thymol has important effects on the survival rate and development time of the insect in the larvae, pupae, and adult stages. While determining the concentrations used in this study, a preliminary study was conducted, and it was determined that the most appropriate range was the concentrations given in the study. Also, these concentrations are depended on the results of previous experiments with *D. melanogaster* and other insects' species, since they allow *D. melanogaster* to complete their development to the adult stage in pre-feeding tests. The fact that thymol, a plant-based chemical, has a negative effect on *D. melanogaster* even at low concentrations creates the idea that this chemical can be used as an insecticide that will create less residue and environmental pollution in nature. However, the safe use of thymol in agriculture requires further research.

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