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# EFFECTS OF TEA FORMS WITH DIFFERENT CAFFEINE CONTENTS ON FECUNDITY AND MORTALITY

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## ABSTRACT

Tea obtained from the leaves of the plant known as *Camellia sinensis* L. is known nowadays as the most consumed beverage after water as a healthy beverage due to its bioactive molecules and high antioxidant capacity.
This study aimed to investigate the effects of six different tea forms (green tea, oolong tea, black tea, white tea, decaffeinated tea, and Pu-erh tea) sold on the market on the larval mortality and egg yield in *Drosophila melanogaster*. The effect of different tea forms on larval mortality rate was tested in Oregon R wild type *D. melanogaster* 3rd stage larvae.
When the obtained data were examined, while the survival percentage in the control group was 95%, the same rate was found in decaffeinated tea. The survival percentage in green tea was determined to be 92%, 83% in oolong tea, 81% in black tea, 74% in white tea, and 39% in Pu-erh tea ($p<0.05$). When the effect of different tea types on egg yield was examined, the highest egg yield was observed in decaffeinated tea and green tea. The lowest egg yield was detected in the flies fed with Pu-erh tea ($p<0.05$).
It was concluded that in the consumption of tea, which is the most consumed beverage after water in the world and Turkey, the amount of caffeine it contains is important, and that attention should be paid to the amount of tea consumed daily, especially due to its adverse effect on fecundity in females.

**Keywords:** Tea forms, *Drosophila melanogaster*, Fecundity, Caffeine, Mortality,

**ÖZET**

*Camellia sinensis* L. olarak bilinen bitkinin yapraklarından elde edilen çay, günümüzde bioyaktif moleküller ve yüksek antioksidan kapasitesi nedeniyle sağlıklı bir içecek olarak sudan sonra en çok tüketilen içecek olarak bilinmektedir.

Bu çalışmada piyasada satışa sunulan 6 farklı çay formunun (yeşil çay, oolong çay, siyah çay, beyaz çay, kafeinsiz çay ve Pu'erh çayı) *Drosophila melanogaster*'de larval mortalite ve yumurta verimi üzerine etkilerinin araştırılması amaçlanmıştır. Farklı çay formlarının larval mortalite oranı üzerine etkisi Oregon R yabanıl tip *D. melanogaster* 3. evre larvalarında test edilmiştir.

Elde ettiğimiz veriler incelendiğinde; Kontrol grubunda yaşam yüzdesi %95 iken kafeinsiz çayda da aynı oran tespit edilmiştir. Yeşil çayda yaşam yüzdesi %92, oolong çayda %83, siyah çayda %81, beyaz çayda 74 ve Pu'erh çayında %39 olarak tespit edilmiştir ($p<0.05$). Farklı çay türlerinin yumurta verimine etkisi incelendiğinde ise en yüksek yumurta veriminin kafeinsiz çay ve yeşil çay olduğu görülmektedir. En düşük yumurta veriminin ise Pu'erh çayı ile beslenen sineklerde olduğu tespit edilmiştir ($p<0.05$).
Dünyada ve ülkemizde sudan sonra en fazla tüketilen içecek olan çayın tüketiminde içerdiği kafein miktarının önemli olduğu, özellikle bayanlarda fekundite üzerine olumsuz etkisi nedeniyle günlük içilen çay miktarına dikkat edilmesi gerektiği sonucuna ulaşılmıştır.

**Anahtar Kelimeler:** Çay formları, *Drosophila melanogaster*, Fekundite, Kafein, Mortalite

## 1. INTRODUCTION

Tea is obtained by processing *Camellia sinensis* leaves grown in tropical and subtropical regions in the world. The tea plant belongs to the Theaceae family and has two species. Of these, *Camellia sinensis* var. *sinensis* of Chinese origin is a small-leaved, bushy plant and grows in many countries with a cold climate in Northeast Asia. The other one *Camellia sinensis* var. *Assamica* is a large-leaved tree grown in the Assam region of India, and it has spread to many countries with a semi-tropical climate. Assamica varieties contain large amounts of tannins and catechins and are especially used in black tea production.

Tea obtained from the leaves of the plant known as *Camellia sinensis* is nowadays described as a healthy beverage due to its bioactive molecules and high antioxidant capacity (Gonzalez et al., 2009). The scientific therapeutic potential of tea has been revealed in recent years. Tea, which is consumed by two-thirds of the world's population, which is the most important beverage after water and which positively affects the human body in many ways, contains more than 4000 chemical substances (Yang and Landau, 2000).

The differences between teas are usually caused by the process, growing conditions, and geographical features. Teas are divided into four groups according to their process or harvesting: black tea (fermented), green tea (not fermented), oolong tea (semi-fermented), and white tea. Green tea is produced from young tea leaves. After withering, steaming or pan-frying, drying, and classification, tea is packed and offered for sale for consumption without being fermented. Pan-frying is necessary to prevent the tea leaves from being fermented by natural enzyme activity. When processing black tea, the tea leaves are fermented for several hours before the processes of either smoke fire, flame fire, or steaming. Withering takes place as the evaporation of water, and a natural fermentation process occurs. The chemical structure of tea leaves changes during fermentation. Unblended teas are named according to country origins or regions (Darjeeling, Assam, China, etc.). Blended teas, on the other hand, are named as type-specific (Earl Gray, Irish Breakfast, etc.) rather than the teas they contain. Unprocessed raw green tea is used as a raw material in the preparation of Pu-erh tea consumed by the Chinese, and the preparation of this tea requires a long fermentation process. The longer preservation process of Pu-erh tea is believed to provide better quality and taste (Wang et al., 2008). Pu-erh tea is a popular beverage, especially due to its special flavor in Southwestern China. It has a lower catechin level than black, green, and oolong tea (Lin et al., 2008).

Oolong and green tea contain high amounts of EGCG (50% to 80% of total catechin) and EGC. However, the amount of these components is lower in black tea. Fresh green tea leaves have 30%-40% catechin in dry weight. Green tea contains about 70% catechin (monomeric flavonoids), 10% minor flavanols (mostly quercetin, kaempferol, myricetin, and their glyco structures), and 20% polymeric flavonoids because some oxidation events occur during the withering process. Of the total flavonoids in green tea, 20% up to 30% can be oxidized to polymers present in black tea. Tea contains many amino acids, but L-theanine (γ-ethylamine-L-glutamic acid) is the most abundant amino acid found in the tea plant as plant-specific and accounts for about 50% of the total amino acid level. Volatile substances in tea contain more than 600 different molecules. Besides, tea contains carbohydrate, caffeine, adenine, gallic acid, tannin, gallotannin, quercetin glycosidase, carotenoids, tocopherols, vitamins (A, K, B, C), low amounts of aminophenyl, and a yellow essential oil, which is solid at 25 °C and has strong aromatic smell and taste (Jayabalan et al., 2008).

Oolong tea is a type of tea that is not recognized in Turkey but of which popularity is increasing all over the world. This tea, which has a different phenolic compound composition than green and black tea, is also noteworthy due to its antioxidant, anticarcinogenic, and antiallergic properties, as well as its diabetes, obesity, atherosclerosis, and heart disease prevention properties (Koca and Bostancı, 2014).

Tea consumed at medium temperature and in certain amounts can be defined as a beverage that positively affects health, in addition to not having any acute or chronic toxic effects. In the studies conducted, it was stated that people who drink tea regularly also have a healthy lifestyle (Schwarz, 1994; Weisburger and Chung, 2002).

The tea composition varies according to the climate, seasons, tea varieties, and age of the tea leaf. Furthermore, the tannins or phenolic substances containing catechin (flavanol) and gallic acid units are at quite high (5-27%) rates in tea (Leung and Foster, 1996). During the fermentation of fresh tea leaves, some catechins are oxidized or condensed to larger polyphenolic molecules (dimers and polymers) such as theaflavins (theaflavin, theaflavin-3-gallate, theaflavin 3' gallate, and theaflavin 3-3' gallate) (3%-6%) and thearubigins (12%-18%). These polymers provide the bitter taste and dark color in black tea. Black tea generally contains thearubigin (70%), theaflavin (12%), flavanol (10%), and catechin (8%). The total polyphenol contents of black and green tea are similar, but this similar content consists of different types of flavonoids depending on the oxidation that occurs during the process (Stangl et al., 2006). A cup of tea prepared with a liter of water and 10 g of tea contains about 300 mg of solid matter. Of this solid matter, 30-40% is catechin, and 3-6% is caffeine (Khan and Mukhtar, 2007).

Studies have demonstrated that tea has different pharmacological effects such as antioxidative, anti-inflammatory, antimutagenic, anticarcinogenic, antiangiogenic, apoptotic, antiobesity, hypocholesterolemic, antiatherosclerotic, antidiabetic, antibacterial, antiviral, and anti-aging (Çelik, 2006). Tea also contains caffeine, in addition to these substances. It should not be ignored that the excessive consumption of the caffeine compound present in the tea content has toxic effects. Due to the adverse effects of caffeine, the tendency to consume caffeine-free products has increased among people (Mazzefera et al., 1991).

*Drosophila melanogaster*, which is called the fruit fly or vinegar fly, is widely used as a model organism in genetic studies. *D. melanogaster* is an invertebrate holometabolous animal from the Insecta class, Diptera order. Immediately after the re-discovery of Mendel's studies, it was considered as an ideal experimental animal for genetic studies in the laboratory of T. H. Morgan in the United

States due to reasons such as small size, the ease of culture, short generation time, abundant breeding, the low number of chromosomes, and huge chromosomes that are advantageous in different studies (Graf et al., 1992). The number of chromosomes is 2n = 8. The maturation period at 25ºC and 60% humidity is 9-11 days (Graf et al., 1992). In the present study, we aimed to evaluate the effect of different types of tea on egg yield and mortality on *D. melanogaster*, which is a frequently used organism in biological studies.

## 2. MATERIAL AND METHODS

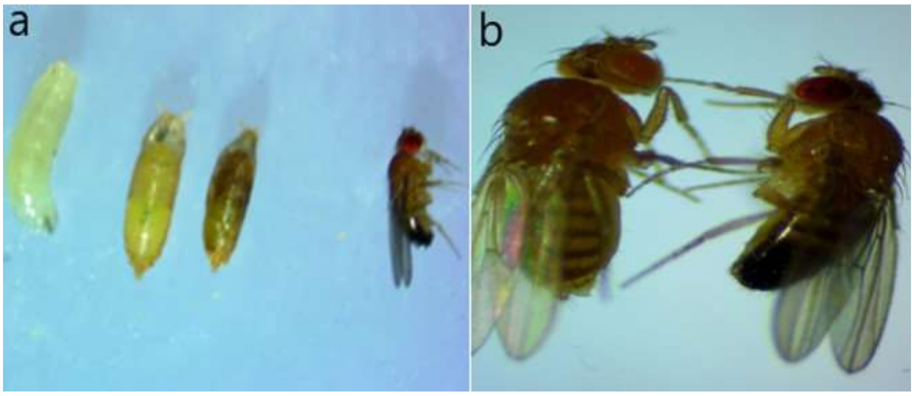
### 2.1. Material

In the study, six different types of tea commercially sold in Turkey were used. These teas were procured from Çaykur and Chado companies. "Black tea," "white tea" and "green tea" were procured from Çaykur, and "oolong tea," "Pu-erh tea," and "decaffeinated tea" were procured from Chado company. The procured teas were preserved closed until the application was performed (Figure 1).


**Figure 1.** Types of tea procured for use in the study

In this study, the Oregon (R) wild type stock of *D. melanogaster* was used to evaluate the effect of different tea types on larval mortality and egg yield in female individuals. *D. melanogaster* Oregon R stock is a wild type stock with normal round, red eyes, and no mutant character (Figure 2).

*D. melanogaster* is reproduced by tailoring for years in the Research Laboratory of the Department of Biology, Faculty of Science and Letters, Amasya University. *Drosophila* stock cultures are kept alive at 40% to 60% relative humidity and 25 ± 1 °C temperature in bottles containing the standard *Drosophila* medium (SDM) in hot cabins with heating and cooling systems always having dark conditions.




**Figures 2 a,b)** Larvae, prepupa, pupa, and mature individuals of *Drosophila melanogaster*

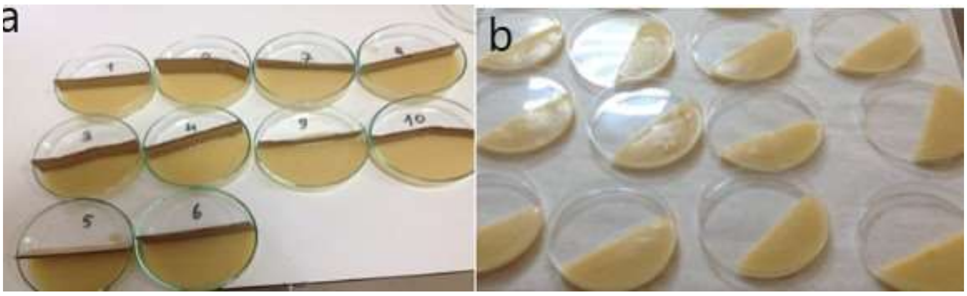
## 2.2. Methods

### *2.2.1. Preparation of experimental sets and the application of tea samples*

During the studies, the instant medium was used in the storage, reproduction, and crossing-over stages of *Drosophila* cultures. The instant *Drosophila* medium was procured from Carolina Biological Supply Company. 1.5 g of *Drosophila* instant medium and 5 ml of distilled water were added to 50 ml Falcon tubes. The teas used in the study were prepared in the form of ml/10 mg stock solutions. By adding 2 g of tea to 200 ml of boiling water, its infusion was waited for 30 minutes, and the infused tea was then transferred to 250 ml bottles for preservation (Figure 3).


**Figures 3 a,b)** Creation of stock solutions by the infusion of different tea types

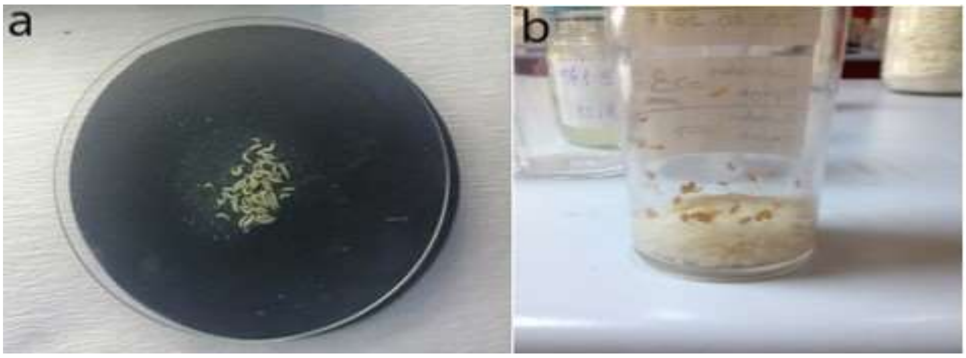
In the present study, the 3rd-stage larvae of *D. melanogaster* were obtained to determine larval mortality, and female individuals of *D. melanogaster* were obtained to determine egg yield. Female and male flies of *D. melanogaster* Oregon R stock were crossed over in 250 ml culture bottles containing SDM, and pre-stocks were prepared. The same aged (1-3 days/72 ± 4 hours) unpaired female flies hatched from the pupa were collected every 5 hours for three days and preserved in culture bottles for use in experiments. The female flies obtained in this way were placed as 3♀♀ X 3♂♂ in the medium prepared in Petri dishes for egg counting (Figure 4).




**Figures 4 a,b)** Experimental setup prepared to determine egg yield

Egg counting was performed for three days, and the data obtained during these three days were averaged. Egg counting was done under a stereomicroscope and in the same time period every day. For three days, the flies were transferred to a fresh medium. The flies were subjected to $CO_2$ etherization during their transfer to the new medium.

Forty female individuals and forty male individuals were placed in nutrient-containing bottles to obtain 3rd stage larvae. These individuals were kept in the same environment for at least one day and allowed to mate. The individuals were then transferred to new bottles containing nutrients and were waited to lay eggs in the medium for 8 hours. Then, the individuals were transferred to other bottles. The goal of an 8-hour egg collection process is to obtain individuals in the same larval stage. The individuals reaching the 3rd larval stage were separated under tap water with the help of fine porous sieves after 72 ± 4 hours. The third stage larvae collected with the help of sieves were transferred to plastic bottles containing the *Drosophila* instant medium, which was wetted by adding 5 ml of freshly prepared tea concentrations to be studied.




**Figures 5a,b)** Determination of the number of individuals that mature in the 3rd stage larvae of *Drosophila melanogaster*

One-two spatula-full (about 100 larvae) larvae were placed on each application medium. By adding 1.5 g medium and 5 ml stock tea solutions to the instant medium, mature flies were waited to hatch (Figure 5). All experiments were carried out with three repetitions.

### *2.2.2. Statistical analysis*

Statistical analyses of the data obtained in the experiments performed to investigate the effects of the researched items on the survival percentage and egg yield were performed using the SPSS (Statistical Package for the Social Sciences) 15.0 program.

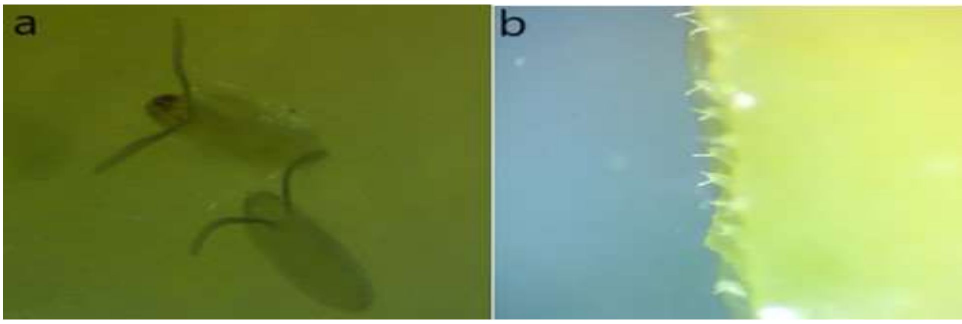
## 3. RESULTS AND DISCUSSION

All experiments in the study were carried out with three repetitions. The results obtained were averaged. The egg yield of the flies in the standard medium prepared in Petri dishes in order to investigate the egg yield was determined under the Isolab stereomicroscope (Table 1) (Figure 6). In the study, the survival percentages obtained as a result of tea application to D.melanogaster 3rd stage larvae are presented in Table 2.

**Table 1**. Data obtained as a result of 3-day egg count in female individuals of *Drosophila melanogaster*

| Teas (ml/10 mg) | First-day egg yield ± SE | Second-day egg yield ± SE | Third-day egg yield ± SE | Mean egg yield ± SE |
|---|---|---|---|---|
| **Control Group** | 65.0±0.57 | 77.0±0.33 | 69.0±1.15 | 70.3±1.52[a] |
| **Oolong Tea** | 55.0±0.33 | 55.0±0.57 | 58.3±0.57 | 56.1±1.10[b] |
| **Black Tea** | 45.0±0.33 | 55.0±1.10 | 54.0±0.33 | 53.0±1.17[b] |
| **White Tea** | 47.0±1.15 | 42.0±2.10 | 57.0±0.57 | 49.0±0.40[b] |
| **Green Tea** | 55.0±0.57 | 67.0±0.33 | 71.0±1.10 | 64.3±1.80[a] |
| **Pu-erh Tea** | 43.0±0.33 | 42.0±0.57 | 44.6±0.33 | 43.0±0.57[c] |
| **Decaffeinated Tea** | 62.0±1.10 | 78.0±1.10 | 56.6±0.33 | 66.0±0.56[a] |

SE: Standard Error; Statistical evaluations of the difference between the groups were made within the group. Values shown with different letters in the same column are significant at the level of $p<0.05$.




**Figures 6a,b**. Determination of egg yield in female individuals of *Drosophila melanogaster* under a microscope

**Table 2.** Survival percentage in larvae as a result of tea application and the number of individuals transformed from larva to pupa and from pupa to mature individuals

| Application Groups (ml/10 mg) | Number of larvae | Transformation from larva to pupa ± SE | Transformation from pupa to mature individuals ± SE | Survival percentage ± SE |
|---|---|---|---|---|
| **Control Group** | 100 | 97±0.58$^{a}$ | 95±0.33$^{a}$ | 95±0.33%$^{a}$ |
| **Green Tea** | 100 | 100$^{a}$ | 96±0.58$^{a}$ | 92±0.57%$^{a}$ |
| **White Tea** | 100 | 82±1.15$^{b}$ | 74±0.58$^{b}$ | 74±0.58%$^{b}$ |
| **Oolong Tea** | 100 | 88±0.33$^{b}$ | 83±1.15$^{c}$ | 83±1.15%$^{c}$ |
| **Black tea** | 100 | 90±0.33$^{a}$ | 81±0.33$^{c}$ | 81±0.57%$^{c}$ |
| **Pu-erh Tea** | 100 | 50±1.15$^{c}$ | 39±1.15$^{d}$ | 39±0.57%$^{d}$ |
| **Decaffeinated Tea** | 100 | 100$^{a}$ | 95±0.58$^{a}$ | 95±0.33%$^{a}$ |

SE: Standard Error; Statistical evaluations of the difference between the groups were made within the group. Values shown with different letters in the same column are significant at the level of $p<0.05$.

In the present study, egg yield in the control group was determined to be 70.3 ± 3.52, as seen in Table 1 and Figure 7. In the experimental groups, while the highest egg yield was found in decaffeinated tea as 66 ± 6.56, the egg yield in green tea was determined to be 64.3 ± 4.8. The lowest egg yield was observed in Pu-erh tea and white tea. It was observed that decaffeinated, green, and oolong teas with the highest egg yield had lower egg yield compared to the control group. Although tea is a very useful

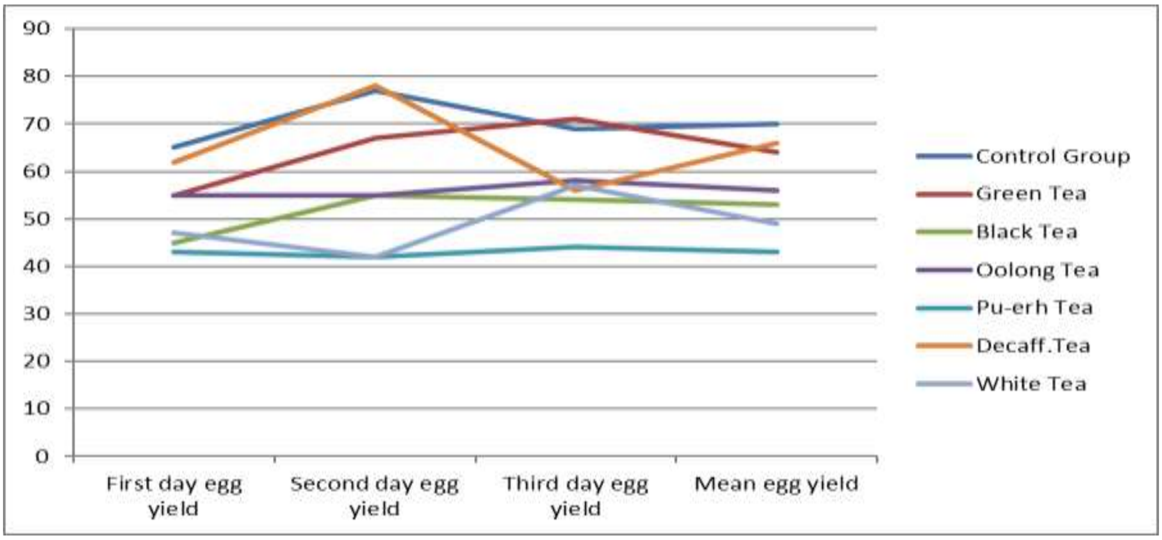



**Figure 7**. Comparison of the 3-day egg yield of female individuals of *Drosophila melanogaster* grown in media containing different types of tea

beverage in terms of the polyphenols it contains, due to its high caffeine content, some types of tea can cause adverse effects, especially in female individuals in terms of egg yield. When the survival percentages given in Table 2 and Figure 8 are compared, the highest survival percentages are observed in decaffeinated tea, green tea, and oolong tea. The number of individuals that transformed from 100 larvae placed in the medium containing tea into pupa and mature was noted.

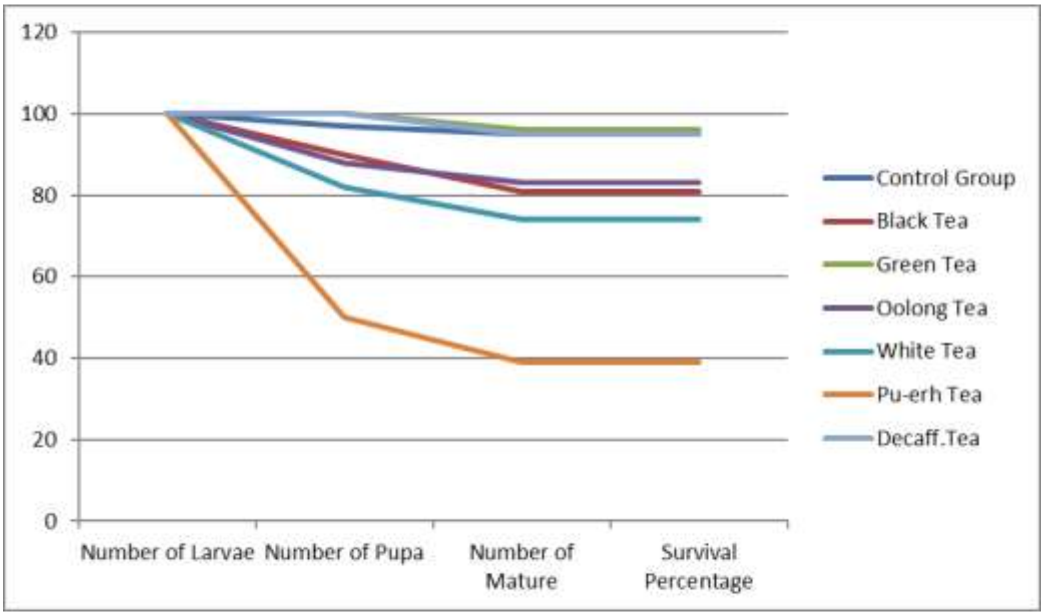



**Figure 8.** Survival percentage in 3rd stage larvae of *Drosophila melanogaster* grown in media containing different types of tea.

When the data obtained as a result of the studies repeated three times are compared with the control group, the decaffeinated tea and the control group are observed to have a 95 ± 0.33% survival percentage. While green tea has a survival percentage of 92 ± 0.57%, oolong and black tea have survival percentages of 83 ± 1.15% and 81 ± 0.57%, respectively. Black tea is followed by white tea, which has a 74% survival percentage. The tea with the lowest survival percentage is Pu-erh tea with 39 ± 0.57%.

## 4. CONCLUSIONS

In the study, it was determined that the egg yield in the experimental groups was lower than the control group. The highest egg yield was found in decaffeinated tea, while the lowest egg yield was observed in Pu-erh tea and white tea in the experimental groups. Although the highest egg yield was observed in decaffeinated, green and oolong teas, egg yield was lower than the control group.

Due to its sensory properties, stimulating effects, and health benefits, tea is one of the most popular beverages in the world. The stimulating effect of tea is due to the caffeine it contains. In addition to the fact that caffeine is a stimulant for the central nervous system, respiration, and heart, it is also a vasodilator (relaxing blood vessels) and diuretic (Jun, 2009). The caffeine content of tea leaves varies according to the type of tea. Its ratio in the dry matter is usually between 2-5% and 150 ml of tea infusion contains about 24-50 mg of caffeine (Jun, 2009).

Although tea is a very useful beverage in terms of the polyphenols it contains, due to its high caffeine content, some types of tea can cause adverse effects, especially in female individuals in terms of egg yield (Hering-Hanit and Gadoth, 2003; Mohanpuria et al., 2010; Nealey, 2019 ). Excessive tea consumption leads to an increase in the level of caffeine besides antioxidant compounds. High levels of caffeine may have adverse effects on health in susceptible individuals (Mohanpuria et al., 2010). Caffeine stays longer in the body in children and pregnant women due to slow metabolization. It is reported that excessive caffeine intake causes infertility in women of childbearing age and birth defects in pregnant women (Miyagishima et al., 2011). Thus, there is a need for the production of tea without caffeine or with reduced caffeine content. In tea processing, a significant reduction of the caffeine content of tea or the removal of caffeine is called decaffeination (Miyagishima et al., 2011).

In a study conducted by Nealey (2019) on the effect of green tea on egg yield in female individuals of *Drosophila melanogaster*, fecundity rates were determined to decrease depending on the increase in tea concentration. Komes et al. (2009) compared five different types of tea, including white, green, yellow, oolong, and black tea in terms of caffeine content. In terms of caffeine content, they ranked teas as white (3.62%)> yellow (3.18%)> black (2.79%)> oolong (2.77%)> green tea (2.35%). They reported that caffeine content varies according to the origin, genetics, environmental factors, harvest time, processing method, and the age of tea leaf. They stated that young shoots contain higher amounts of caffeine. Therefore, the caffeine content of white tea made from young shoots is higher in comparison with others. In our study, in decaffeinated tea, higher values were found in both egg yield and survival percentage compared to all other teas. We are thinking that the factor affecting egg yield is the different caffeine ratios in different tea ingredients. Ranking according to the caffeine contents in the study of Komes et al. supports our findings.

The number of individuals that transformed from 100 larvae placed in the medium containing tea into pupa and mature was noted and survival percentages were compared. When the data obtained as a result of the study repeated three times are compared with the control group, the decaffeinated tea and the control group are observed to have a survival percentage (95 ± 0.33%). Green tea, oolong tea, black tea and white tea follow them in decreasing proportions, respectively. The tea with the lowest survival percentage is Pu-erh tea with 39 ± 0.57%.

In addition, since black tea and Pu-erh tea are more exposed to fermentation processes, their catechin content, which positively affects health, is lower than other teas (Lin et al., 2008; Zuo et al., 2002). In our study, egg yield values were found to be low and mortality rates were the highest in these two tea groups. We think that these effects may depend on the catechin amounts. This hypothesis needs to be supported by prospective studies.

In conlusion, tea is the most consumed drink in the world after water. Therefore, people should know the caffeine content in the tea they drink. When daily tea consumption is 10 cups and more, caffeine intake reaches dangerous levels. Moreover, it can also cause negative effects for people, with the consumption of other foods containing caffeine. People should mostly consume teas with low caffeine content or decaffeinated teas. Based on the available pieces of evidence, the use of caffeine in pregnancy is recommended to be reduced to below 300 mg per day. Although it is reported that a moderate amount of caffeine taken daily does not cause any adverse effects in the infant, the amount of caffeine recommended to pregnant women is 125 mg/day. Therefore, people should be aware of the caffeine content in beverages and foods.

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