The Effect of UV Radiation and Fruit Feedings (Banana and Guava) on the Survival Rate and Morphological Changes of Reproductive Organ of Fruit Fly (*Drosophila melanogaster* Meigen, 1830)

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ABSTRACT
The increasing ozone concentration in the upper stratosphere was not significant enough to be able to protect from the detrimental effect that ultraviolet (UV) light radiates. One solution to counter this problem is by using antioxidants as protectants, such as those contained in fruits. In order to elucidate that ability, experiments can be done using fruit fly (*Drosophila melanogaster*) as the animal model. It is commonly used because it has physiological and pharmacological similarities with humans. This study aims to elucidate the effect of guava, which has a high amount of antioxidant, as a feeding medium on the survival rate and morphological changes of the reproductive organs of fruit flies after certain periods of UV exposure. The fruit fly culture was obtained from the Genetic Laboratory, Faculty of Biology, UGM. The experiments were performed with 2 hours of UV exposure and 2 feeding mediums (banana and guava) and a control (without UV exposure) also with 2 feeding mediums (banana and guava). Reproductive organs were isolated from 4 various treatments. Observations were made on the survival rate and morphological changes of the reproductive organs from the F1 generations. The results showed that UV exposure was capable of suppressing survival rate at larval stage by 44.38% (fed with banana) and 48.01% (fed with guava); at pupal stage by 57.55% (fed with banana) and 47.83% (fed with guava); and at the imago stage by 60.00% (fed with banana) and 13.93% (fed with guava). The UV exposure showed impacts on the morphology of the reproductive organs, and as UV protectant, guava gave better results compared to banana.

Keywords: *Antioxidant, D. melanogaster, Protectant, UV.*

1. INTRODUCTION

Ozone in the stratosphere acts as a protective shield against UV radiation [1]. Thus, decreasing ozone concentration means more UV exposure to the earth and its inhabitants. It has been reported that there is a significant increase of the upper stratospheric ozone layer since the year of 2000 [1]. However, there is still no evidence found on the increase in the total column of ozone since 1998, which implies that the lower stratospheric layer has not been increasing as much as the upper [2], [3]. On the contrary, there is actually a report found on the increase of the lower stratospheric ozone layer by using Chemistry Transport Model (CTM) [4]. However, it is found that CTM could not provide

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insight into the underlying dynamical driver of the long term ozone decline or growth. It is also stated that the data provided by Chipperfield in 2017 was actually 60% larger than what was observed [3]. The increase in 2017-2018 did enhance the magnitude of the stratospheric ozone layer, but the recovery as of quasi global (60ºS-60ºN) still displayed a reduction trend since 1998 [3]. Hence, from this speculative data and uncertain reports, it can be assumed that the detrimental effect of UV radiation still persists.

One of the effects of UV radiation is indirect amplification of free radical concentration in aerobic organisms including humans. Free radicals, in this case the reactive oxygen species (ROS), are actually naturally occurring in aerobic organisms as an intermediate product of oxygen reduction process to become water molecules. However, it is reported that UV radiation generates singlet oxygen molecules by interacting with photosensitizer (e.g. flavins and porphyrins) in such orchestrated ways that the flavin becomes excited and acts as electron donor for the ground-state-level oxygen, which is the non-reactive di-radical species with the same spin upwards of two unpaired electrons [5]. It is also reported that UV-B, one the three types of UV lights, is able to generate ROS by reacting with catalase [6]. Thus, it is proven that UV light is absolutely capable of amplifying reactive radicals in the human body. These radicals are actually important, because they act as signaling molecules through a variety of mechanisms [7]–[9]. However, if the homeostasis feedback mechanism could not handle the increase in radicals, they will immediately react with nearby molecules, and damage DNA, protein, as well as lipid [10]–[12].

The negative effect of excessive ROS could also affect reproductive organs since it is reported that the germinal sperm cells are sensitive to oxidative stress due to the lack of cytoplasmic defenses. Moreover, sperm contains polyunsaturated fatty acids, which are vulnerable to ROS, as it is also lacking in DNA repair mechanisms [13]. ROS were also reported to affect female reproductive organs, in a related study, when performing a morphometric on follicles to observe atresia in resting follicles, it is showed that in progressive atresia, the mitochondrial membrane and the oocyte nuclear were both ruptured. It was assumed that the early involvement of mitochondria suggests that the damage is induced by ROS, because mitochondria is known as the site of oxidative phosphorylation which generates radical intermediates [14]. Hence, it can be assumed that progressively increasing UV-induced oxygen radicals will affect the reproductive organs' morphometric.

The homeostatic mechanisms rendering the excessive ROS are called enzymatic-antioxidants which is done by scavenging or neutralizing the ROS [15]. Thus, introducing the non-enzymatic antioxidants would likely help the system as well. One of those so-called non-enzymatic antioxidants is vitamin C, which is already popular or available in fruits such as tangerine, kiwi, orange, and many more. Vitamin C is reported to render the free radicals chain reaction by scavenging the initiating radicals and stops the detrimental effect of those over-produced ROS [16]. In a related study, it is shown that guava (*Psidium guajava*) has a high amount of vitamin C that is 491.6 mg/100 g [17]. Hence, it makes it as a good candidate for a protectant or a recovery agent.

Fruit fly is the most commonly used animal model due to its similarities in pharmacological and physiological with humans. It is reported to be used as good animal model for antioxidant therapy in Parkinson's disease [18]. It is also used as an animal model for sperm interaction with the female reproductive tract, and it is reported that nearly 75% of disease-related genes in humans have functional orthologs in this fly species [19]. Besides, it has a short life cycle which makes it easy to handle at a low cost and in a small container. Therefore, fruit flies can also be assumed to be a good animal model for this study. Hence, this study aims to elucidate the effect of a certain dose of UV exposure on the survival rate and morphology of reproductive organs (male and female) of *D. melanogaster*. This study also aims to study the antioxidant ability of guava and banana to protect *D. melanogaster* after certain periods of UV exposure.

2. MATERIALS AND METHODS

2. 1. Obtaining the fruit fly culture

The main culture of Fruit Fly was obtained from the Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada. The obtained culture was the acclimated wild type phenotype and was fed on banana as the standard feeding medium. The obtained culture was in a jar bottle and was used as the main source for the rearing culture.

2. 2. Fruit fly rearing in banana and guava feeding medium

The mass rearing of fruit flies was done in Entomology Laboratory, Faculty of Biology, Universitas Gadjah Mada. The rearing was performed using the Hodson and Chiang method [20]. Bottle jar

was used as the rearing container. The bottle jar was sterilized using 70% ethanol by wiping the entire surface of the bottle using a tissue that had been moistened with the ethanol. Banana and/or guava was mixed with Tapai using a 6:1 ratio, then sodium benzoate was added later, all of the mixing was done using a blender until they homogenized. The mixed medium of 20 ml was inserted to each bottle jar which will be used for sexing (24 pieces) and another 20 ml which will be used for rearing (12 pieces). A piece of folded paper was vertically added on top of the feeding medium as a place to lay eggs. The bottle was then closed using a clear plastic that had been perforated and then placed at room temperature until the medium solidified. The isolated virgins were placed in a new bottle jar.

2. 3. Setting the UV exposure treatment on fruit flies

The treated groups of virgin fruit flies were exposed to UV light (*Bossecom* TL 2; 10 watt). The unpaired *D. melanogaster* were exposed for 4 days, 2 hours each day (12.00 - 14.00 Western Indonesia Time), and then simultaneously for 3 days with the same dose after they were paired. Eventually, the number of individuals of each stage (larva, pupa and imago) of the F1 generation were counted and tabulated. The control group was reared in a separate room, the number of individuals of each stage (larva, pupa and imago) were also counted and tabulated. The experiments were done in triplicates.

2. 4. Observing and measuring male and female reproductive organs

The reproductive organs were isolated from 3 pairs of the treated fruit flies, each from the banana and guava feeding medium. The other 3 pairs were isolated from the untreated fruit flies, each from the banana and guava feeding medium. The isolation method was done according to Zamore and Ma [21]. To observe the reproductive organs clearly, an object glass was placed on black-colored background. The dissection was done using saline solution and dissection needles. The collected fruit flies were euthanized by chloroform, then transferred to a petri dish. Each fruit fly was placed under a light microscope and the saline solution was dropped. The fruit flies were positioned on their abdomen facing the top, so that the position of the reproductive organs was consistent between right (dextral) and left (sinistral). The dissection was performed by separating the 6th abdomen segment (A6) from the 7th segment (A7) carefully. The isolated testicles and ovaries were measured (length and width, in millimeter) using a digital microscope (*supereyes* 250x). The experiments were done in triplicates.

2.5. Data analysis

The data obtained from the experiments were analyzed using formulas and statistical analysis. Survival rate of the treated and control groups were graphed in the survival rate bar plot as a result of using Equation (1) and (2):

$$\text{Survival rate} = \left(\frac{Treated}{Control}\right) x\ 100\ =\ SR \quad (1)$$

$$\text{UV light suppression} = 100\% - SR \quad (2)$$

The morphometric of the reproductive organs were analyzed by one-way ANOVA, continued by Tukey and T-Test with significance of $p \leq 0.05$ [22]. Morphology characteristics of reproductive organs were also analyzed in a descriptive manner. The statistical analysis was performed using SPSS software v. 25.

3. RESULTS AND DISCUSSION

The treated and the control group was compared using the formulas which resulted in the UV exposure effect on each stage of fruit flies at 2 different feeding mediums. The result is shown below (Figure 1.).

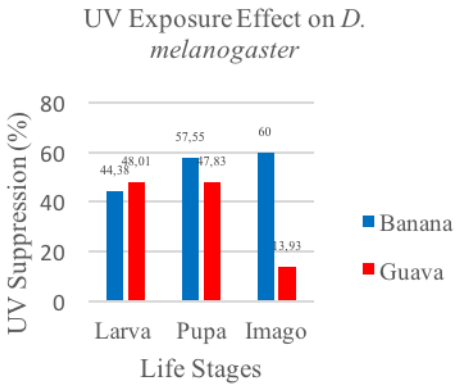

Figure 1. The capability of UV radiation in suppressing *D. melanogaster* life stages: larval stage 44.38% (banana) and 48.01% (guava); pupal stage 57.55% (banana) and 47.83% (guava); and imago stage 60.00% (banana) and 13.93% (guava). Higher percentage indicates a higher suppression.

The UV suppression shows effects to each stage of *D. melanogaster* (Figure 1.) at varying degrees. It generally shows that UV exposure could suppress all of the life stages of fruit flies. The banana feeding

medium group is relatively more suppressed at each stage, except for the larval stage. It is assumed that this was due to the feeding medium condition. The guava feeding medium, which has more water content of 83.37±2.4% [23], may affect the larval development and also affect the place to lay eggs. It is reported that the female fruit flies prefer a dry condition to lay its offspring [24].

However, in the next stage, the pupal stage of the banana feeding medium is more suppressed compared to its larval stage and the guava feeding medium (Fig 1.). It can be assumed that the UV exposure may actually affect the fruit fly larvae fed with banana feeding medium more than the guava ones, despite the UV suppression percentage which shows a higher suppression for guava (48.01%) than banana (44.38%). This may happen due to the banana feeding medium group could not contain the increasing rate of ROS in their body which eventually affect their growth and development by disrupting the molecules that are important in this particular process [25] which leads to problems in their pupal development. This statement is supported by a finding which showed that larval stage development affects later stage [26]. It is also reported that ROS actually has its counterintuitive effect as second messenger ROS is able to extend *Drosophila* lifespan [27]. However, this phenomenon was not observed in this study, hence the sole effect was only the detrimental ones.

The UV suppression on the imago stage was shown to heavily suppress fruit flies fed on banana (60% suppression) (Fig 1.), while the fruit flies fed with guava feeding medium was only suppressed by a small percentage (13.93%) comparatively. This can be explained due to the complexity of the fruit flies imago feeding on guava medium which results in their capability to handle the ROS effect more properly by using the vitamin C obtained from the guava as well as the fully developed enzymatic antioxidant from their body. Therefore, this synergistic mechanism was achieved. On the other hand, the imago of fruit flies fed with banana feeding medium accumulate the ROS through their life stages which may disturb the development of their enzymatic antioxidants [28].

The results in the following tables were produced by statistical analysis on the reproductive organs measurements of male and female *D. melanogaster* fed with banana and guava feeding medium compared to the exposed and the control group. T-test (Table 1.) and (Table 2.) was performed to validate the significant difference on each individual group of treatment, in this case the difference between UV treated group and the control group without taking consideration to its feeding medium, likewise when to test each feeding medium group, the UV effect was neglected.

Table 1. T-Test on male reproductive organs of fruit flies

Parameters	Mediums	
	Banana	Guava
Dex. Length (mm)*	2.37±0.15a	2.27±0.28a
Sin. Length (mm)*	1.91±0.34a	2.34±0.35a
Dex. Width (mm)*	0.16±0.02a	0.19±0.04a
Sin. Width (mm)*	0.34±0.50a	0.16±0.03b
Parameters	**Treatments**	
	UV	non UV
Dex. Length (mm)*	2.37±0.19a	2.28±0.26a
Sin. Length (mm)*	2.06±0.14a	2.19±0.40a
Dex. Width (mm)*	0.17±0.05a	0.18±0.03a
Sin. Width (mm)*	0.34±0.49a	0.16±0.04b

*)same letter on the same row indicates that there is no significant difference.

Table 2. T-Test on female reproductive organs of fruit flies

Parameters	Mediums	
	Banana	Guava
Dex. Length (mm)*	0.82±0.17a	0.98±0.13a
Sin. Length (mm)*	0.86±0.18a	0.92±0.18a
Dex. Width (mm)*	0.51±0.11a	0.62±0.11a
Sin. Width (mm)*	0.58±0.08a	0.55±0.06a
Parameters	**Treatments**	
	UV	non UV
Dex. Length (mm)*	0.85±0.8a	0.96±0.14a
Sin. Length (mm)*	0.75±0.11a	1.02±0.11a
Dex. Width (mm)*	0.54±0.10a	0.59±0.14a
Sin. Width (mm)*	0.55±0.08a	0.58±0.06a

*)same word on the same row means there is no significance difference.

The T-Test results show a non-significant difference in general for each treatment, except for the sinistral width of testicles from fruit flies fed with guava feeding medium and the sinistral width of testicles from fruit flies treated without UV exposure. This suggests that fruit flies fed with guava feeding medium have narrower sinistral width of testicles. It

Table 3. Tukey's post hoc test on male reproductive organs of fruit flies

Parameters	Treatments			
	Banana+UV	Guava+UV	Banana non UV	Guava non UV
Dex. Length (mm)*	2.32±0.19a	2.42±0.21a	2.43±0.09a	2.13±0.30a
Sin. Length (mm)*	1.76±0.38a	2.35±0.13a	2.06±0.27a	2.32±0.53a
Dex. Width (mm)*	0.15±0.03a	0.19±0.06a	0.17±0.01a	0.20±0.03a
Sin. Width (mm)*	0.52±0.71a	0.15±0.04a	0.15±0.06a	0.18±0.01a

*)same letter on the same row means there is no significant difference.

Table 4. Tukey's post hoc test on female reproductive organs of fruit flies

Parameters	Treatments			
	Banana+UV	Guava+UV	Banana non UV	Guava non UV
Dex. Length (mm)*	0.81±0.27a	0.89±0.06a	0.84±0.04a	1.08±0.10a
Sin. Length (mm)*	0.75±0.17a	0.76±0.05a	0.97±0.13ab	1.07±0.06b
Dex. Width (mm)*	0.55±0.16a	0.54±0.01a	0.48±0.03a	0.70±0.12a
Sin. Width (mm)*	0.60±0.10a	0.51±0.01a	0.57±0.07a	0.59±0.06a

*) same letter on the same row means there is no significant difference.

is assumed that the energy obtained that should have been allocated for reproductive development is allocated instead to increase their resistance to ROS [29]. In other ways, it can also be assumed that the energy obtained from the bananas is allocated solely for reproductive development. However, further study should be conducted to confirm this statement.

The T-Test also showed a contradictory result which is a narrower sinistral testicle of fruit flies treated without the UV exposure. It is contradictory because in theory, it is reported that high amounts of ROS could disrupt nearby molecular structures as well as functions [30]. Hence, it can be inferred that there could be other variables that may affect this result, for example a swelling caused by the ruptured cells that may have been damaged by the increasing ROS amount [31]. However, this result should also be elucidated further due to its vagueness. From these non-significant results, it can also be assumed that the feeding medium is a capable recovery agent for the fruit flies. This can be seen from the results of the measurements that were not varied that much.

On the other hand, the Tukey's (Table 3.) and (Table 4.) was done to see the significant difference of each treatment combination as a whole. The Tukey's post hoc resulted in a coherent result with the hypothesis, despite only 2 measurements gave significant results. The measurement on the reproductive organs after the combination of treatment showed a higher length of sinistral ovary both for the banana and guava fed fruit flies without the UV or in other ways, the UV exposure shortened the sinistral ovaries from both feeding medium. This suggests that UV exposure has indeed amplified the

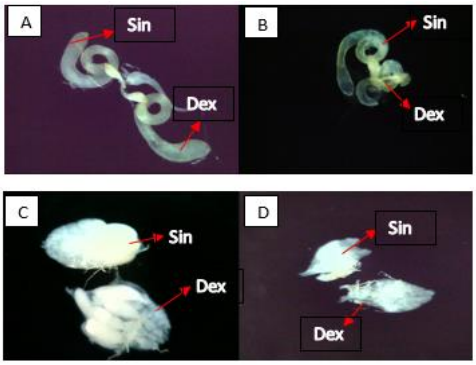

Figure 2. Reproductive organs of male and female of *D. melanogaster*: A) Testis (non-UV), B) Testis (with UV irradiation), C) Ovaries (non-UV), and D) Ovaries (with UV irradiation).

ROS concentration [32]. Therefore, the uncontrolled rise of ROS concentration disrupts the ovaries' development which eventually change their morphology. Nonetheless, the UV exposure was proven to have an impact on the reproductive organs the non-significant result also suggested the capability of the feeding medium (guava) as a recovery agent.

The Figure 2. were isolated from 4 various groups, the observation was performed using

Supereyes digital microscope with a magnification of 250x.

The visual observations were also made on the sinistral and dextral portion of both reproductive organs (Fig 2.). Using visual observation, the control group (A & C) showed a larger dimension compared to the treated group (C & D). From this visual observation, it can be assumed that the UV exposure has an impact on the reproductive organs, since it shows a different dimension compared to the control group. Through this visual observation, the ROS is assumed to be capable of reducing the size of both male and female reproductive organs or causing disruptions to its development in such a way that it is different from the control [33], [34]. Despite being disrupted by the ROS, the reproductive organ is still capable to develop and operate fully, as this is assumed due to the synergistic recovery effects of non-enzymatic antioxidants as well as the vitamin C obtained from the feeding medium (guava) and enzymatic antioxidants [16].

Based on the results of this research, it can be concluded that the UV exposure was capable of suppressing survival at larval stage by 44.38% (in banana fed group) and 48.01% (in guava fed group); at pupal stage by 57.55% (in banana fed group) and 47.83% (in guava fed group); and at the imago stage by 60.00% (in banana fed group) and 13.93% (in guava fed group). The UV exposure showed an impact on the morphology of reproductive organs, and as UV protectant, guava was found to be a better fruit feeding medium compared to banana.

AUTHORS' CONTRIBUTION

The research was designed by H.A and I.S. The data was collected by H.A. The paper was written by H.A, N.K, and I.S.

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