The impact on wild type strain using a disinfectant for growth medium in *Drosophila melanogaster*

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Abstract

When exposed to harsh environmental conditions, such as chemical toxin or low temperature, the development of *Drosophila melanogaster* (fruit fly) will be affected. Fruit flies have many suitable features for relevant study. They are relatively easy to differentiate the sexes and to obtain virgin males and females for genetic crosses. The anesthesia can be performed with unsophisticated equipment. Flies have a short life cycle and grow well at room temperature at a lower cost. Propionic acid as a disinfectant is added to the growth medium preventing bacteria or fungi infection during culture process. Little is known about the effect of acid on flies' development. This study aimed to explore the influence of growth dates, dry weight and body length in wild strain in different propionic acid concentrations. The growth days became distinctive when flies reached pupa and adult stages. They grew in 0.8% propionic acid and control treatments better than those in 1.3% and 1.8% propionic acid treatments. It indicated that propionic acid might play a role in affecting flies' development. The flies' total number, day weight and body length were measured and analyzed using Analysis of Variance. The results showed that there was no significant difference in the treatments because the probabilities of all the F-test were greater than standard 0.05. This was attributed to large standard deviations in the treatments. In accordance with the averages of the traits, flies developed in vials containing 0.8% to 1.3% propionic acid better than those in vials with 1.8% propionic acid or those in control (no propionic acid). Further study was proposed to learn more about the effect of propionic acid on flies.

Introduction

Drosophila melanogaster had a common name, fruit fly. It was typically used in physiology studies due to its short life cycle, large number of offspring per generation, and lower cost to culture in a regular biology lab. The fruit fly was developed as an *in vivo* model organism for toxicology studies, in particular, the field of nanotoxicity (Ong et al., 2014). Staats et al. (2008) evaluated *Drosophila melanogaster* as a versatile model organism in experimental food and nutrition research and made suggestions for future research directions. Kim et al. (2020) demonstrated how environmental temperature and macronutrient balance combine to affect key life-history traits related to fitness and to mediate trade-offs among these traits. Rocco et al. (2022) found that temperature had mixed impacts on weight and length in females and males of the wild type strain, apertous and bar eye mutants.

Propionic acid is a short-chain fatty acid that is the main fermentation product of the enteric microbiome. It is used as a disinfectant and associated with health issues. Wang et al. (2017) reported that *D. melanogaster* female adults displayed an oviposition avoidance to propionic and butyric acids in a dosage-dependent manner. Propionic acid dramatically delayed the developmental duration of their progeny and reduced the survival rate in a dosage-dependent manner, and 2% propionic acid caused death of larvae. Demir et al. (2023) pointed out that significant genotoxic effects were detected in selected cell targets in a concentration dependent manner, especially at two highest concentrations (5 and 10 mM) of propionic acid.

The objectives of the study were (1) to identify differences in growth dates at the stages of first egg, larva, pupa, and adult fly of wild type strain in different concentrations of propionic acid; (2) to examine the effects on production, weight, and length of adult flies.

Materials and Methods

Fruit fly strain

The wild type strain was purchased from Carolina Biological Supply Company and maintained in the biology lab.

Concentration treatments

There were three different concentrations of propionic acid added to the medium. and control (without propionic acid in the medium). Treatment 1: no propionic acid, Treatment 2: low concentration (0.8%), Treatment 3: mediate concentration (1.3%), Treatment 4: high concentration (1.8%). We mixed 6 g of the medium with 30 ml of distilled water containing a concentration of propionic acid in a vial. We created three vials for the measurements in each treatment. All flies grew at 21°C.

Sexing flies

It is quite easy to tell males from females. Males are generally smaller and have a darker and more rounded abdomen. The black and gray on abdomen is the easiest feature to recognize. In addition, males have tarsal sex combs on their first pair of legs. These are black and very distinctive but can only be seen under relatively high magnification.

Culture medium

Instant Drosophila medium from Carolina Biological Company needed neither cooking nor sterilizing. It contained a blue coloring agent to facilitate observation of larvae. An equal volume of instant Drosophila medium (one inch of culture medium) and 0.8% propionic acid solution is added to a vial. Then a few grains of dry viable yeast were sprinkled on top. After one minute, flies could be introduced, and the vial plugged. Add 0.8 ml, 1.3 ml, and 1.8 ml of propionic acid to 100 ml dH_2O (autoclaved) to make 0.8%, 1.3%, and 1.8% propionic acid solution, respectively.

Fruit fly handling

To cross the flies, FlyNap (an anesthesia agent) was soaked on the end of a wand. The wand was then inserted into the vial containing the F_1 generation of flies, in a manner which allowed none of the flies to escape. The flies were monitored to determine when FlyNap should be removed from the vial once fully anesthetized. The process of anesthetizing flies took around 2 minutes. Caution was taken in order to avoid overexposure to FlyNap which is lethal to flies in excessive dosage.

Propagation of flies

After flies were fully anesthetized, the cap of the vial was removed, and flies were transferred onto a white card. They were then placed under a dissecting microscope to identify sexual features. Once the sex of each fly was identified, five males and five females were placed into a vial containing culture media. The vial was lying on the side to ensure flies did not get stuck to culture medium for a day. After flies recuperated from FlyNap, the vials were placed upright. In four days, the parental generation of flies was removed from the vial. The larvae developed into mature flies within 10 to 20 days. Upon the emergence of the next generation, mature flies were ready to be counted and scored under a dissecting microscope according to their inherited traits.

First emergency of egg, pupa, and adult fly

The dates for first egg, pupa, and adult fly emerged were recorded.

Adult fly collection and tally

The adult flies were placed on a standard commercial medium in a vial and allowed oviposition. After we saw the eggs, we discarded the parental flies. Upon the defined time point, we collected the adult flies, counted the number of flies in each vial and recorded as total number of flies.

Dry weight measurements for adult fly

To determine weight, flies were anesthetized, transferred into pre-weighed 1.5-mL tubes. Then they were placed and dried in an oven at 60°C with tube lids open. In 24 hours, we measured weight in the tubes on an analytical scale. We performed three biologically independent repeats in each concentration treatment.

Body length measurements for adult fly

For adult flies, their lengths were measured under the microscope using Motic Images Plus 2.0 software. We performed three biologically independent repeats in each concentration treatment. In a repeat, ten flies were selected to be measured.

Statistical analysis

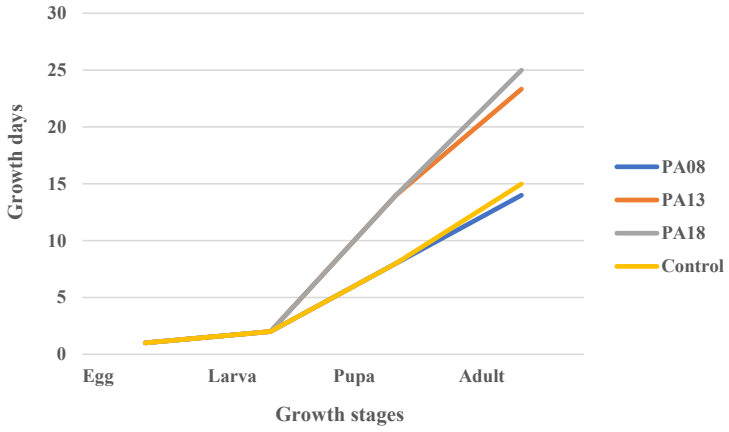
Variance of analysis (ANOVA) was performed using Data Analysis in Excel (Brase, 2023). The post hoc Tukey Honestly Significant Difference (HSD) test was conducted by an online test calculator for comparing multiple treatments (Vasavada, 2016).

Results

The difference in the growth days in four treatments

Figure 1

The growth days recorded from the mating dates in four treatments



In Figure 1, we could see that the growth periods of egg and larva overlapped in all the treatments. At pupa and adult stages, the growth became distinct. For the treatments of 0.8% propionic acid and control, the flies matured about ten days earlier than those in 1.3% and 1.8% propionic acid treatments. It was obvious that the medium and high concentrations prolonged the number of days of fly growth starting at pupa stage until adult stage.

One-way ANOVA for total number, weight, and length of flies in four treatments

Table 1

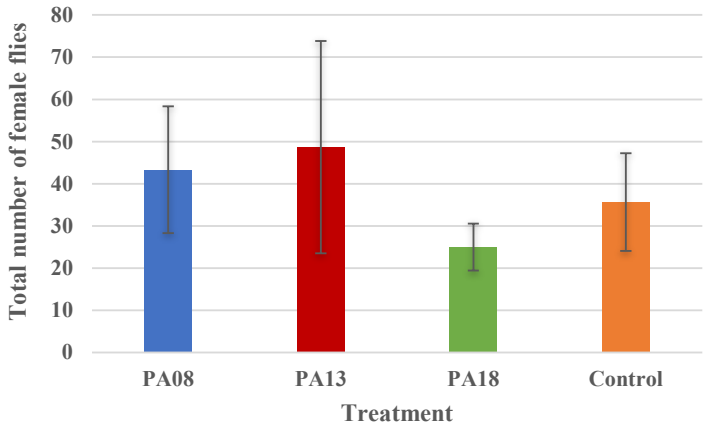
One-way ANOVA for total number of female flies in four treatments

Source of variation	SS	df	MS	F	P-Value	F critical
Between treatments	949.7	3	316.6	1.24	0.359	4.07
Within treatments	2050.0	8	256.3			
Total	2999.7	11				

According to the F test in ANOVA (Table 1), there was no significant difference in total number for female flies among four treatments because the probability was 0.359 much larger than standard one 0.05. It meant the concentration of propionic acid might not greatly affect the overall production of female flies.

Figure 2

Total number of female flies in four treatments



In Figure 2, the 0.8% and 1.3% propionic acid treatments had a higher production from 42 to 49 flies per vial compared to the other two treatments. The lowest production, 22 flies per vial, came from 1.8% propionic acid treatments. The standard deviation for each treatment was relatively large.

The F test showed in Table 2 that it was not significantly different in weight of female flies among four treatments due to the probability more than 0.05. It indicated that the propionic acid treatments did not alter the weight of female flies dramatically.

In Figure 3, the weight of female flies (0.25 g per fly) with a high standard deviation was the heaviest one in 0.8% propionic acid treatment. The weight from the other three treatments seemed close to 0.2 g per fly.

Table 2

One-way ANOVA for weight of female flies in four treatments

Source of variation	SS	df	MS	F	P-Value	F critical
Between treatments	0.0097	3	0.0032	3.16	0.086	4.07
Within treatments	0.0082	8	0.0010			
Total	0.0178	11				

Figure 3

Weight of female flies in four treatments

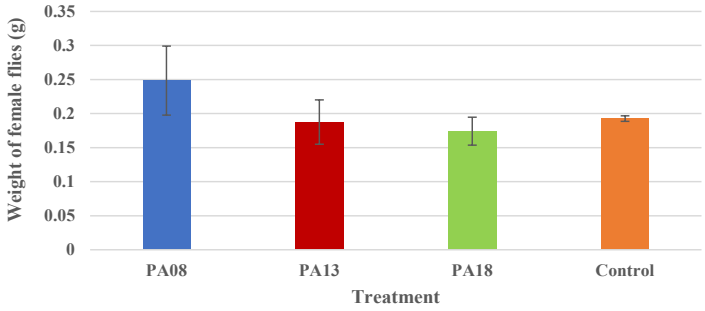


Table 3

One-way ANOVA for length of female flies in four treatments

Source of variation	SS	df	MS	F	P-Value	F critical
Between treatments	3816.1	3	1272.0	0.266	0.848	4.07
Within treatments	38283.4	8	4785.4			
Total	42099.5	11				

The F test (Table 3) demonstrated the insignificant different outcome in length of female flies in four treatments owe to a large probability (0.848) larger than 0.05. The result pointed to the tendency that the propionic acid might not change the length obviously.

From Figure 4, the length of female flies varied from about 2230 μm in 1.8% propionic acid treatment to 2250 μm in 1.3% propionic acid treatment, which presented a large standard deviation.

In Table 4, the results of the F test revealed that total number of male flies was not significant in four treatments at 5% level because

of the probability being 0.346 more than 0.05. In general, it illustrated that the propionic acid did not affect the production of male flies noticeably.

Figure 4

Length of female flies in four treatments

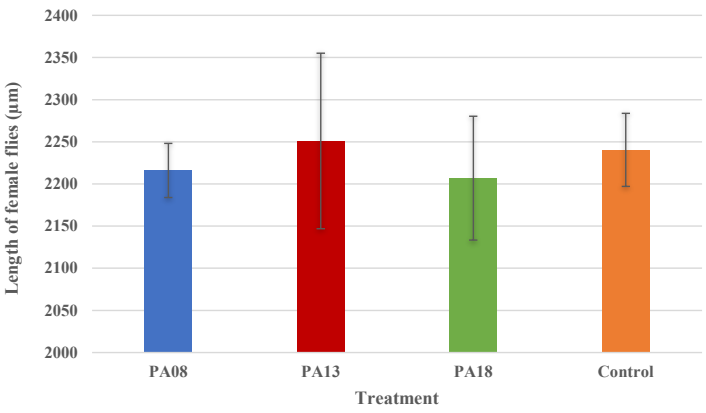


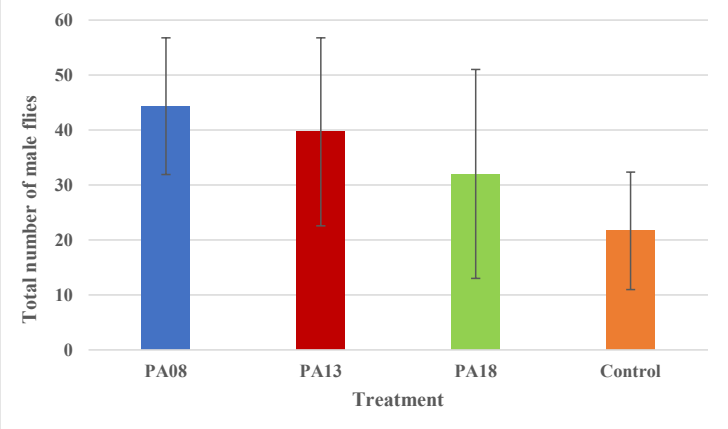
Table 4

One-way ANOVA for total number of male flies in four treatments

Source of variation	SS	df	MS	F	P-Value	F critical
Between treatments	882.9	3	294.3	1.28	0.346	4.07
Within treatments	1844.0	8	230.5			
Total	2726.9	11				

Figure 5

Total number of male flies in four treatments



From Figure 5, we can see that 0.8% propionic acid had a highest production around 44 flies per vial. The next one was 40 flies per vial in 1.3% propionic acid treatment. The control treatment had the lowest production, 21 flies per vial. The standard deviations were large for all the treatments.

Table 5

One-way ANOVA for weight of male flies in four treatments

Source of variation	SS	df	MS	F	P-Value	F critical
Between treatments	0.0046	3	0.0015	2.74	0.113	4.07
Within treatments	0.0045	8	0.0006			
Total	0.0091	11				

The F test in Table 5 showed that no significant difference was present in four treatments in terms of weight of male flies. The probability was 0.113 more than 0.05. It indicated that propionic acid did not influence weight of male flies among the treatments evidently.

Figure 6

Weight of male flies in four treatments

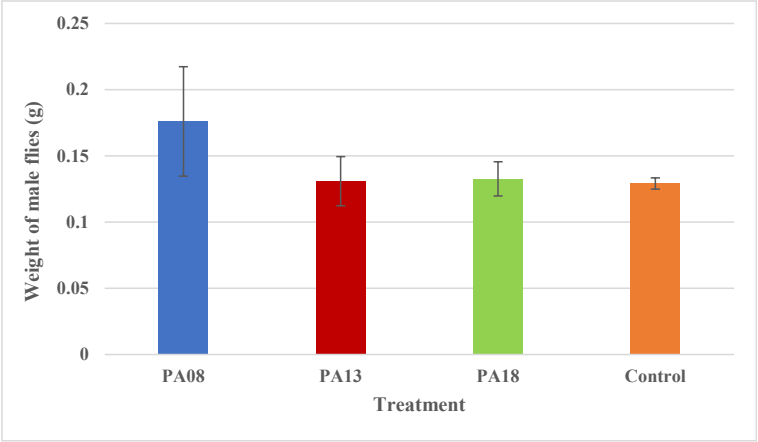


Table 6

One-way ANOVA for length of male flies in four treatments

Source of variation	SS	df	MS	F	P-Value	F critical
Between treatments	12996.4	3	4332.1	1.11	0.400	4.07
Within treatments	31233.5	8	3904.2			
Total	44230.0	11				

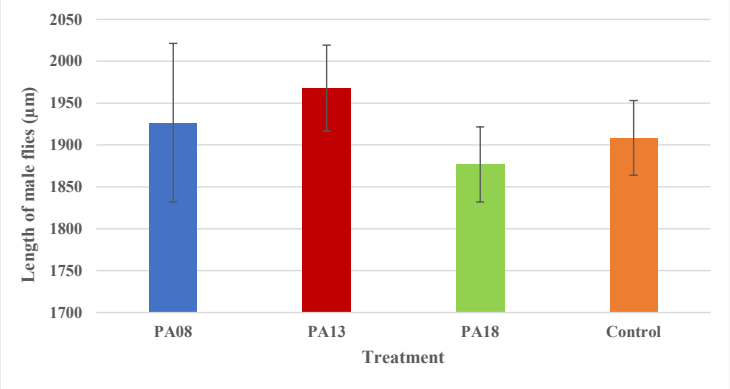
In Figure 6, the 0.8% propionic acid treatment had the highest weight, about 0.18 g per fly with a large standard deviation. The

weights from other treatments were close to one another.

In Table 6, the result of F test for length of male flies in four treatments exhibited the similar tendency, which there was no significant difference among the treatments because the probability (0.4) was much lower than 0.05. It elucidated that propionic acid did not modify length of male flies in the treatments distinctly.

Figure 7

Length of male flies in four treatments



The 1.3% propionic acid treatment had the longest length of male flies, 1970 μm. Whereas, the shortest length, 1880 μm, came from the 1.8% propionic acid treatment. The standard deviations in all the treatments were relatively large.

Conclusion

The flies grew at a constant speed over egg and larva stages in all the treatments. However, it changed when they reached pupa and adult stages. The growth days were shorter in the 0.8% propionic acid treatment and control than those in the 1.3% and 1.8% propionic acid treatments. In terms of total number, weight, and length of the flies, all the results of F-test shared the same trend, which was no significant difference among the treatments demonstrating the propionic acid did not affect those traits apparently. Part of the reason could be attributed to large standard deviation in each treatment. Overall, the flies grew in the vials containing 0.8% to 1.3% propionic acid better than those in the vials with 1.8% propionic acid or control treatment. A future study may include observing the developmental process of flies at individual stages and detecting differences due to addition of propionic acid in the growth medium of fruit fly.

Acknowledgments

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