JOURNAL OF
EMERGING INVESTIGATORS

Article

Parental exposure of cannabinoids THC and CBD reduces reproductive rates in *Drosophila melanogaster*

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SUMMARY

The FDA discourages women from using marijuana during pregnancy or breastfeeding on the basis that cannabinoids such as THC (delta-9-tetrahydrocannabinol) and CBD (cannabidiol) can negatively affect fetal brain development. However, despite increasing accessibility of cannabinoids, their impact on fertility, pregnancy, and reproduction rates has not been well studied. We hypothesized that CBD and THC would reduce reproductive rates in *Drosophila melanogaster*. Our experiments were designed to show how cannabinoids might impact hatch rates in *Drosophila* by exposing them to previously reported concentrations of CBD and THC. We did a hatch assay with control, THC, and CBD groups to compare rates of hatching between controls and flies treated with either 0.5 mg/mL THC or 0.5 mg/mL CBD. Compared to control groups, both cannabinoid groups showed a statistically significant decrease in reproductive rates. The control group had a hatch rate of 94.8%, while the CBD and THC groups had hatch rates of 86.2% and 89.6%, respectively. In the THC group, pupae were noted to turn red and die before emerging. While we predicted cannabinoids would reduce reproductive rates, we had expected THC would have the greater impact on reproduction. The results from our experiments indicated that CBD showed a greater reduction on hatching than THC. However, THC caused unusual and unexpected mortalities. This study provides evidence that cannabinoids such as THC and CBD reduce the rate of reproductive success, although the exact mechanism requires further study.

INTRODUCTION

Marijuana is a psychoactive plant that has been used for centuries, originating in central Asia. Marijuana and its derivatives are used for their medicinal properties that help with anxiety, anorexia, and pain (1). In the US, medical use of marijuana and cannabis products is legal in 38 states, with a 2019 survey showing that approximately 18% of Americans have used cannabis at least once (2).

The psychoactive and medicinal properties of marijuana can largely be attributed to cannabinoid compounds. Cannabinoids bind to cannabinoid receptors in the brain and thereby impact the body's endocannabinoid system, which facilitates nerve synaptic communication (3). Cannabinoids can be extracted from the cannabis plant or made synthetically. Common ways to ingest cannabinoids include smoking the plant, consuming cannabinoid oils, consuming cannabinoid edibles, or inhaling vapor with cannabinoids.

There are six types of cannabinoids, of which THC (delta-9-tetrahydrocannabinol) and CBD (cannabidiol) are the most commonly encountered. THC is the psychoactive component of cannabis and gives a "high" because it binds with receptors controlling pain and emotions (1, 4). CBD, on the other hand, reduces feelings of anxiety and the negative effects of THC such as paranoia, xerostomia, and impairment of cognitive functions. Although cannabis has many reported positive effects (1), there are also many potential neurological repercussions of its use. Cannabis has been linked with memory loss and memory issues, especially for people who use it before their brain fully develops (5). THC blocks receptors that impact neuron communication, which impairs the ability to create new neural pathways (5).

Marijuana and its derivatives also affect the reproductive system. Although mice treated with THC demonstrated no effects on sperm motility, testis shape or reproductive success (6), smoking marijuana was associated with a 29% reduction in total sperm count in human males (7). THC has also been found to disrupt ovulation cycle lengths in rhesus monkeys (8). Furthermore, THC can cause a significant decrease in fetal growth in human pregnancies (9). Another cause for concern is that THC is never fully degraded and stays stored in body fat, from which it can be passed on to the baby through breast milk (10).

Drosophila melanogaster is a model system which has been widely used to study teratogenicity and the reproductive impacts of toxins (11). *Drosophila* has been increasingly popularized in the toxicology field for molecular mechanisms. They are particularly useful due to their quick life cycle and high yield of offspring (12). This species has also been used to demonstrate the impact of hemp seed meal, a marijuana-related product, on development and reproduction (13). Here, we sought to discover further evidence on how marijuana's main cannabinoids, CBD and THC, may affect reproductive rates. Due to *Drosophila's* role as a model organism, the impact of cannabinoids on *Drosophila* may help predict the impact of cannabinoids on humans.

Here we report how THC and CBD at previously reported doses (17) decrease reproductive rates in *Drosophila melanogaster*. We performed a hatch assay with a control group, a CBD-treated group, and a THC-treated group. We found the hatch rate of the control group was 94.8%, the THC group was 89.6%, and the CBD group was 86.2%. There was a rare abnormality with the THC group in which some pupae would turn red and die. The study found that both the THC and CBD groups had statistically significant lower hatch

https://doi.org/10.59720/24-080

rates compared to the control group, providing evidence that cannabinoids decreased reproductive rates in *Drosophila melanogaster*. Similarly, long term use of THC and CBD in humans can negatively affect fertility and cause complications with pregnancy.

RESULTS

To quantify measures representing the impact of a treatment on the fertility of *Drosophila* we compared hatch rate (**Figure 1**). The hatch rate is the amount of successfully emerged *Drosophila* larvae divided by the number of eggs. The control group had a hatch rate of 94.8%, which was greater compared to the THC group, with a hatch rate of 89.6%, and the CBD group, with a hatch rate of 86.2% (**Table 1**). Because we examined categorical data (hatched vs unhatched), we used a chi-squared test to compare the hatch rate of each cannabinoid group to the control. Both conditions were statistically significant, as the CBD group had a p-value of 0.00000353 and the THC group had a p-value of 0.0022 (**Figure 2**). The THC group took longer to emerge from their eggs, mostly emerging on day 12, while most of the control group emerged on day 9 or day 10. The THC group also had some red pupae that melted and died with time (**Figure 3**). Pupae are the third stage of the *Drosophila* life cycle and are naturally a small brown oval about the size of an adult *Drosophila*. These abnormal pupae were very rare, occurring in only 5 out of the 500 eggs, and none survived.

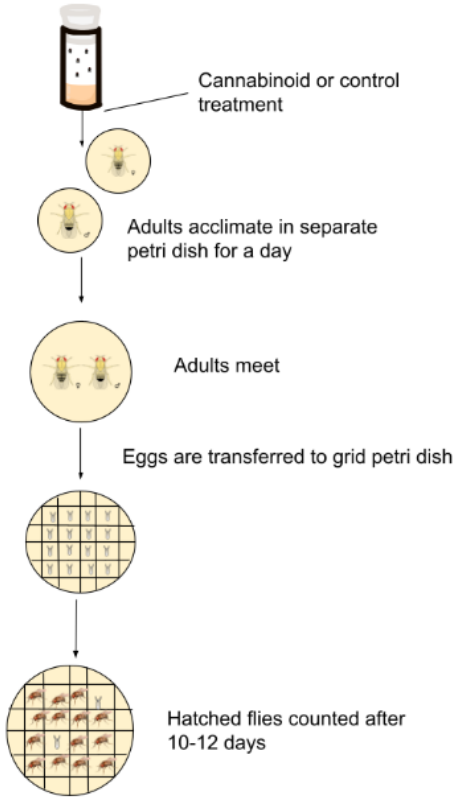

Figure 1: Model showing the hatch assay experimentation process. *Drosophila* were treated with either normal or cannabinoid infused media then acclimated in untreated agar petri dishes prior to copulation with similarly treated *Drosophila*. Eggs were moved to a new petri dish, and emerged *Drosophila* were counted after 10-12 days.

DISCUSSION

This study joins several previous studies that have found that cannabinoids produce a biologically significant decrease in reproductive rates in model organisms (6-9, 13). Due to these findings and the relative paucity of studies, the FDA recommends against the use of cannabis products containing cannabinoids THC and CBD during pregnancy and breastfeeding (18). Moreover, the FDA site does not address marijuana or cannabinoid use in men and women seeking or likely to experience pregnancy.

We hypothesized that cannabinoids would decrease reproduction in *Drosophila* because CBD reduces motor activity in *Drosophila*, and THC negatively affects reproductive and developmental systems in other organisms (16). Specifically, we expected THC to have a greater reduction in reproductive rates than CBD due to the stigma that THC causes infertility and harms human conception and fetal development (18).

The results of THC and CBD treatment showed a statistically significant decrease in reproduction rates compared to the control. While the difference in reproduction rates was statistically significant, which could be evidence that THC and CBD decrease reproduction in *Drosophila*, it is hard to conclude if the results are biologically significant. CBD treatment showed an 8.6% decrease ($p = 0.00000353$) from control with a relatively mild dose (0.5 mg/mL), which could potentially be biologically significant. Future studies could experiment with different doses to further examine biological significance.

The THC-treated group had a lower percentage decrease in reproduction rate of 5.2% ($p = 0.0022$) which may not necessarily indicate biological significance when looking at the percentage alone. However, the THC group uniquely developed red pupae that would die. This phenomenon was exclusive to our experiment and has not been observed in similar experiments using this model system. While the decrease in reproductive rate with THC treatment was statistically significant, it didn't reduce hatch rates by a large noticeable difference. One factor may have been that infusing the food with the THC was not effective, and so the *Drosophila* parents may have been underdosed. Further experiments should use liquid chromatography to confirm the dosage of the cannabinoids. The quantitative data neither convinces nor disproves whether THC has a biological significant reduction of reproductive rates, but the qualitative data, such as the red pupae, create suspicion that THC may impact the development of *Drosophila* in the pupae stage.

	Treatment		
	Control	CBD	THC
Hatched	474	431	448
Unhatched	26	69	52
Hatch Rate %	94.8	86.2	89.6
p-value compared to control		0.00000353	0.0022

Table 1: Number of Drosophila melanogaster hatched and unhatched per treatment. Hatch rate percentages were calculated using n=500 eggs. P-values were calculated using a chi-squared test compared to the control group.

https://doi.org/10.59720/24-080

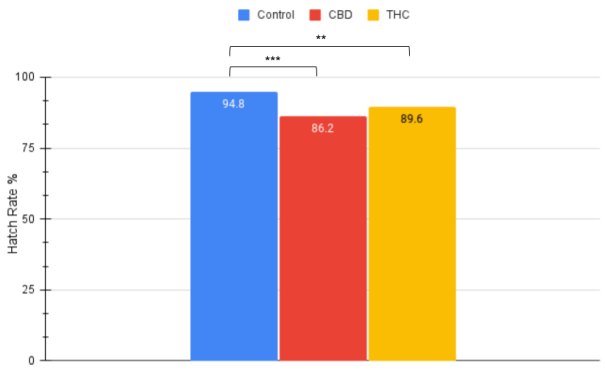

Figure 2: *Drosophila* hatch rate with and without cannabinoids. *Drosophila* virgins were given cannabinoid treated media at a concentration of 0.5mg/ml. Hatch rate percentages reflect the number of eggs that emerged per treatment group (n=500). Chi-squared test, (**p < 0.01, *** p < 0.001)

Figure 3: Red *Drosophila* melanogaster pupa. A rare bright red pupa from THC group. Virgin adults were treated with 0.5mg/ml THC media and allowed to copulate. Eggs were counted and moved to gridded plate to be observed.

We were surprised that CBD resulted in a larger percent decrease in reproductive rates compared to THC, as THC is more widely known to negatively impact the reproductive process in fertility and development (6, 7, 18). In future studies, it may be more informative to test CBD and THC together to simulate what the average human consumes, as the combination of THC and CBD may have a stronger or unique effect. THC delayed the time it took for the eggs to emerge, noticeably slowing the development of *Drosophila*, but CBD had a larger decrease in reproductive rates. Hence, it would be hard to conclude from our data which cannabinoid is more impactful on reproduction.

While our data clearly shows that cannabinoids can decrease reproduction rates, the reduction may be so minor that it might not be of much concern. Generally, scientists accept that cannabinoids such as THC and CBD decrease reproduction by reducing sperm count or harming embryo development (6, 7, 9). Pregnant mothers are advised to stay away from marijuana use which may relate to the red pupae that died before emerging. Marijuana use during pregnancy may harm the development of the child after birth due to the early exposure to THC. Other work has found that CBD and THC reduce sperm count, which may explain the difference in reproduction rates compared to the control in this study (7). In an observational study looking at marijuana use and infertility, CBD and THC use correlated with lower male fertility rates, but since we used more than one male fly for each group, we were not able to examine whether any individual male flies showed infertility (6, 11).

One potential limitation with our experiment was that we infused the *Drosophila* food source by boiling crystalline concentrate. This may not give accurate dosing with the THC, because the compound is not water-soluble and does not fully mix with the food. Relatedly, we began with crystalline THC-a, which does not become THC unless exposed to high temperatures. If some THC-a did not react to the heat and become THC upon boiling, this could also lead to an underdosage. These considerations may explain why THC had a lower reproductive rate decrease than CBD when other studies have shown a more dramatic negative effect of THC on reproductive systems.

In this study, we showed a statistically significant decrease in the reproductive rates of *Drosophila melanogaster* exposed to previously reported doses (17) of CBD and THC. Our results support current FDA recommendations against CBD and THC use during pregnancy and breastfeeding. These results also suggest a need for caution among people planning or at risk for pregnancy. Future studies could use different methods to treat the insects, experiment with different dosages of CBD and THC, and examine the copulation process under their influence.

MATERIALS AND METHODS

Drosophila Organism Model

We used wingless *Drosophila melanogaster* from Josh's Frogs, Freshly Started Wingless *Drosophila melanogaster* Fruit Fly Culture.

Hatch Assay

Ten breeding vials of wild-type, flightless *Drosophila* were left to copulate at 25°C for 1-2 days, after which the adult flies were removed. After 10-12 days, *Drosophila* flies would emerge from the eggs. We used the 8-hour method twice a day for 2-3 days to collect virgin flies. *Drosophila* are unable to copulate until 8 hours after they emerge, so removing all the adults and collecting virgins again after 6 hours will ensure the *Drosophila* are virgins. We collected at least 90 female and 45 male flies for use in the drug treatment experiments.

THC and CBD Treatment

We infused the THC into the food by adding crystalline concentrate to the boiling water before adding the dehydrated *Drosophila* food. We infused the CBD by adding CBD concentrate oil to the boiling water before mixing with the fly food. We put *Drosophila* in vials with infused media 0.5mg/ml of THC or CBD. We split virgin females into vials of 10 and gave them either control food, THC food with a final dose of 0.5 mg/ml or CBD food with a final dose of 0.5 mg/ml. Doses were based on a previous study working with cannabinoids and *Drosophila*, in which the midrange dose was 0.5mg/ml (17).

Collection of Flies for Reproductive Evaluation

Males were split up into vials of five receiving the same treatments as the females. Equal amounts of flies were assigned to each treatment. *Drosophila* were housed with

https://doi.org/10.59720/24-080

infused media for three days, after which we moved the *Drosophila* to a new untreated agar plate for one day to acclimate. We grouped 2-3 males and 6-9 females with same treatment and allowed them to copulate for three days. We then removed the adults and placed the eggs on a new gridded agar plate. We counted the amount of emerged *Drosophila* per agar plate and summed the total for each treatment group. Each treatment had a total of 500 eggs, with each trial having about 100-150 eggs.

Statistical Analysis

A chi-squared test was used to compare the hatch rate percentages of the CBD and THC groups to the control to determine if the differences were statistically significant (α=0.05). O_i is the observed number of emerged *Drosophila* in a specific treatment group divided by n, which was 500 per treatment. E_i represents the expected number of emerged adults and was the control hatch. $\chi^2 = \sum(O_i - E_i)^2/E_i$

ACKNOWLEDGMENTS

We would like to acknowledge Dr. Paloma Vargas and Oxnard College for allowing us to use lab equipment for the experimentation process. We would also like to acknowledge Nila P for serving as a mentor throughout our research. We also want to thank Maria for helping us publish our research.

Received: February 23, 2024
Accepted: June 28, 2024
Published: September 19, 2024

REFERENCES

1. Peter Grinspoon, MD, "Medical Marijuana", *Harvard Health Publishing*, 10 Apr. 2020, www.health.harvard.edu/blog/medical-marijuana-2018011513085. Accessed 31 Jan. 2024.
2. "Data and Statistics," *Centers for Disease Control and Prevention*, 8 June 2021, www.cdc.gov/cannabis/data-research/facts-stats/?CDC_AAref_Val=https://www.cdc.gov/marijuana/data-statistics.htm. Accessed 31 Jan. 2024.
3. De Pierto, MaryAnn, "Endocannabinoids: What Are They and What Do They Do?" *Medical News Today*, MediLexicon International, 27 Feb. 2021, www.medicalnewstoday.com/articles/endocannabinoid Accessed 31 Jan. 2024.
4. Ashton, John C and Michelle Glass, "The cannabinoid CB2 receptor as a target for inflammation-dependent neurodegeneration." *Current neuropharmacology* vol. 5, no. 2, 2007, pp. 73-80, doi:10.2174/157015907780866884
5. "What are marijuana's long-term effects on the brain?" *National Institute on Drug Abuse*, 17 Apr. 2023, nida.nih.gov/publications/drugfacts/cannabis-marijuana Accessed 5 Feb. 2024.
6. López-Cardona AP, et al. "Effect of chronic THC administration in the reproductive organs of male mice, spermatozoa and in vitro fertilization." *Biochemical Pharmacology,* vol 157, Nov. 2018, pp 294-303. doi.org/10.1016/j.bcp.2018.07.045
7. Ilnitsky Sara and Stan Van Uum, "Marijuana and fertility." *CMAJ*. Vol 191, No 23, Jun 2019, E638. doi:10.1503/cmaj.181577
8. Asch, R H, et al. "Effects of delta 9-tetrahydrocannabinol during the follicular phase of the rhesus monkey (Macaca mulatta)." *The Journal of clinical endocrinology and metabolism,* vol. 52, No 1, 1 Jan 1981, pp 50-55. doi.org/10.1210/jcem-52-1-50
9. El Marroun H, et al. "Intrauterine cannabis exposure affects fetal growth trajectories: the Generation R Study." *J Am Acad Child Adolesc Psychiatry.* vol 48, No 12, Dec 2009, pp 1173-1181. doi: 10.1097/CHI.0b013e3181bfa8ee.
10. "Know the Risks of Marijuana", *Substance Abuse and Mental Health Services Administration*, 27 Feb. 2023, www.samhsa.gov/marijuana. Accessed 31 Jan. 2024.
11. Mojica-Vázquez LH, et al. "Mercury chloride exposure induces DNA damage, reduces fertility, and alters somatic and germline cells in *Drosophila melanogaster* ovaries." *Environ Sci Pollut Res Int.* vol 26, No 31. Nov 2019, pp 32322-32332. doi.org/10.1007/s11356-019-06449-4
12. Rand, MD, et al. "Developmental toxicity assays using the *Drosophila* model." *Curr Protoc Toxicol*. Vol 59, No 1. 20 Feb 2014 pp 1-20. doi: 10.1002/0471140856.tx0112s59.
13. Lee MJ, et al. "Dietary hempseed meal intake increases body growth and shortens the larval stage via the upregulation of cell growth and sterol levels in *Drosophila melanogaster*." *Molecules and Cells*. Vol. 30, No 1, Jul 2010, pp 29-36. doi: 10.1007/s10059-010-0085-0.
14. Plummer, Danielle Raiman. "Three CBD Recommendations That May Ease Pregnancy Nausea and Vomiting." *HG Pharmacist*, 30 Dec. 2023, hgpharmacist.com/cbdrecommendations/. Accessed 31 Jan. 2024.
15. Malkin, Alex. "CBD for a Pregnant Woman Is It Safe?" *CBD.Market*, 4 Jan. 2020, cbd.market/cbdblog/cbd-for-a-pregnant-woman-is-it-safe#Is-CBD-Safe-When-Pregnant? Accessed 31 Jan. 2024.
16. Casciato, S, et al. "The Effect of Cannabidiol (CBD) on the Short-Term Memory of Young *Drosophila melanogaster*." *Journal of Biological Sciences*, jbs.camden.rutgers.edu/index/volume-6-2020/effect-cannabidiol-cbd-short-term-memory-young-drosophila-melanogaster/. Accessed 31 Jan. 2024.
17. He, J, et al. "Cannabinoids modulate food preference and consumption in *Drosophila melanogaster*." *Scientific Reports,* vol 11 No. 1, 25 Feb 2021, pp 4709. doi: 10.1038/s41598-021-84180-2.
18. "What You Should Know about Using CBD When Pregnant or Breastfeeding," *U.S. Food and Drug Administration*, 16 Oct. 2019, www.fda.gov/consumers/consumer-updates/what-you-should-know-about-using-cannabis-including-cbd-when-pregnant-or-breastfeeding. Accessed 31 Jan. 2024.