Effects of Natural and Synthetic Pesticides on *D. rerio* Locomotion

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Abstract

Pesticide pollution is a serious problem in the world, particularly in places with a close proximity to the ocean. When these chemicals inevitably leak into groundwater or runoff, humans and animals alike can be exposed to them and subsequently put at an increased risk of neurotoxic responses, birth defects, and cancer. This study sought to determine whether or not natural alternatives to pesticides–in particular, neem oil–might have lesser effects on aquatic life and human nervous systems than synthetic pesticides. In order to do so, larval *D. rerio* (≤7dpf), a species commonly used as a model organism for nervous system function, were separated into three different treatment groups: one was treated with a 20 µg/L concentration of Sawyer Insect Repellent (active ingredient permethrin), one was treated with the same concentration of neem oil (natural pesticide), and one was left untreated to act as a control group. Short videos of these groups were recorded daily for three consecutive days, after which time the movement patterns in the videos were tracked and analyzed. It was determined that exposure to synthetic pesticides caused a significant (p value <0.01) increase in the distance traveled by the *D. rerio*, suggesting a neurotoxic response. The fish that were placed in the neem oil solution were notably harmed as well, exhibiting body axis curvature accompanied by an inability to swim straight forward; by the second day, all fish in the neem oil group had died. These findings suggest that neem oil as a natural pesticide might actually be just as detrimental to human and *D. rerio* nervous systems as compared to synthetic pesticides, if not even worse. This study could provide a basis for further investigation into alternatives to conventional pesticides, and demonstrates the alarming fact that "natural" doesn't necessarily mean "safe".

Introduction

Pesticides are substances or mixtures that are used to repel, kill, or otherwise mitigate any pests, and primarily target those that are destructive to crops (What Is a Pesticide? | US EPA, 2014). These chemicals are essential for any farmer to use in order to maximize crop yield, but problems arise when a process known as "pesticide drift" inevitably occurs. This term denotes the movement of pesticide droplets from their intended location, often into water sources or other nearby environments. When this happens, wildlife and humans alike are put at risk, particularly in areas with large expanses of farmland that require mass quantities of pesticides to be applied to them (El Afandi & Irfan, 2024).

A large-scale study performed in France several years ago involved researchers who bought and prepared commercial food products according to survey results that dictated the most common foods in the area. Each of these dishes was then tested for pesticide residue. Upon analyzing the data, it was found that an astonishing 37% of tested foods contained remnants of one or more pesticides, the most common of which were those that are applied to crops post-harvest (Alexandre Nougadère et al., 2012). Even if pesticides don't leak into runoff, failure to properly clean crops can result in humans consuming them regardless, as shown by the aforementioned research. Whether we know it or not, pesticides are all around us–including the foods we eat.

As one can imagine, exposure to chemicals that are capable of killing pests can have similarly harmful effects on other animals, humans included: a number of studies have shown correlations between pesticide exposure and medical issues such as neurotoxic responses, birth defects, and even cancer (Alarcon et al., 2005; Bjørling-Poulsen et al., 2008). This makes the question of how to mitigate these effects more pressing than ever, especially when an estimated 5.6 billion pounds of pesticides are applied each year across the globe–and as many as 25 million agricultural workers experience accidental pesticide poisonings over that same time frame (Michael, 2009).

As pesticide pollution has become an increasingly well-known issue, “natural” or “organic” alternatives have begun to appear. A 2023 evaluation of scientific studies claimed that these alternatives, including but not limited to neem oil (derived from a tree oil) and pyrethrins (extracted from chrysanthemums), are significantly less toxic than their chemical counterparts (Burtscher-Schaden et al., 2022). The review noted that the percentages of natural pesticides that had been shown to have harmful effects on aquatic life, humans, etc. were significantly lower than those of synthetic pesticides.

While organic pesticides may be slightly less effective in managing pests than the typical synthetic ones, a study performed by Adnan and colleagues demonstrated that this difference is only slight. Researchers tested the efficacy of three synthetic pesticides (imidacloprid, endosulfan, and cypermethrin) and one natural pesticide (neem oil) on managing groups of mango hoppers, a pest that often plagues mango farms throughout southeast Asia. The results showed that while imidacloprid was significantly more effective than the rest, the third most effective pesticide tested (which still produced a roughly 60% drop in the population of mango hoppers) had an effectiveness comparable to that of neem oil (Adnan et al., 2014). Because of its functionality, neem oil was chosen as a natural insecticide to be tested in this study.

However, while chemical pesticides have been proven to be linked to a variety of negative health effects, the concrete extent of their impact is hard to determine as a result of one glaring limitation: for obvious reasons, researchers cannot test directly on humans. Due to this, as Sanborn and colleagues put it, “The major limitation of studies of the health effects of pesticides is their inability to demonstrate cause-effect relationships” (Alarcon et al., 2005). In the hopes of combating this limitation, this study will make use of the model organism *D. rerio* (commonly known as zebrafish) in order to test the effects of different kinds of pesticides on human and animal locomotion. This species is commonly used for such purposes, as their nervous systems function quite similarly to humans’; thus, the findings of this study can be extended to creatures beyond just *D. rerio*.

Although there is no shortage of studies looking into the effects of pesticides on *D. rerio*, as of yet, none have investigated the potential impacts of natural/organic pesticides on the species. Most have instead been focused on proving pesticides to be harmful rather than investigating any potential solution to said harm. So far, most research merely encourages agricultural workers to apply pesticides in moderation, as was suggested by Garud Aishwarya and colleagues, who published a systematic review of pesticides' toxicity, sustainability, and environmental impact (Garud et al., 2024); unfortunately, this so-called solution is far from satisfactory. Thus, the purpose of this study was to evaluate the potential for natural pesticides to work as a safer alternative to traditional synthetic substances.

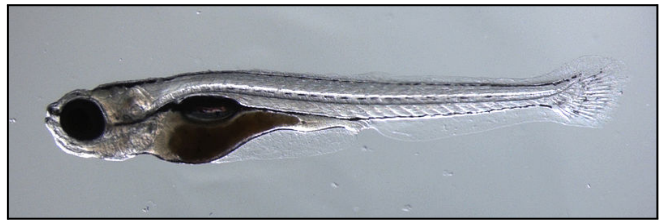


Figure 1. A close-up view of a larval *D. rerio* (zebrafish), such as those used in this study (*How Zebrafish Can Help the Science of Neurodegeneration*, 2018)

Additionally, movement patterns and locomotion are a lesser studied aspect of *D. rerio* health. While other scientists have looked into the developmental and cardiac implications of pesticide exposure in the species, research into how it may impact their nervous system function is scarce (Ferry Saputra et al., 2023; Tai et al., 2021). Since increased or decreased locomotor activity are known signs of neurotoxicity in *D. rerio*, measuring the distance traveled by the fish was determined to be a practical way to gain crucial insight into the consequences of pesticide exposure (d'Amora & Giordani, 2018). Based on this information, the research question was formulated: To what extent do synthetic pesticides affect *D. rerio* locomotion as compared to natural alternatives?

Drawing on the aforementioned studies that established the apparent neurotoxicity of synthetic pesticides, it was hypothesized that exposure to synthetic pesticides would cause *D. rerio* activity to drop significantly, while neem oil would only slightly lower the distance traveled by the fish. While natural pesticides are indeed believed to be less harmful than their chemical counterparts, they are still likely to have at least some negative effects on aquatic life (Helmut Burtscher-Schaden et al., 2022). This fact informed the development of the hypothesis, and it was theorized that impeded nervous system function would primarily be demonstrated by a decrease in locomotor activity.

Research Design

For this study, an experimental design was utilized to obtain the desired data. This was the most practical design since this would allow for definitive comparisons to be drawn between the different groups of *D. rerio*, thereby streamlining the process of data analysis. As previously mentioned, the subjects of this experiment were juvenile *D. rerio* (≤7 dpf), which are an ideal model organism for human nervous systems (Doszyn et al., 2024). In order to comply with ethical regulations, the *D. rerio* used in this study were no longer experimented on after reaching the age of 7 days post-fertilization; additionally, care was taken not to subject the animals to any unnecessary stress outside of that which was necessary to the study.

Before starting the experiment, several methods of containment were considered for the *D. rerio*, but 90mm petri dishes were ultimately used for their practicality. Their size allowed *D.*

rerio enough space to move around, making it easy to track their movements, while still being able to fit within the camera's field of view. Additionally, a shallow water level was ideal to ensure that the majority of the movement tracked occurs in the 2D plane, allowing for accurate measurements of the distance traveled by each fish (Widrick et al., 2023).

Methods

A solution of 20 μg/L Sawyer Permethrin Insect Repellent was prepared prior to the commencement of the study, alongside a solution of the same concentration of 100% pure cold-pressed neem oil. For the purposes of this study, the insect repellent (containing an active ingredient of 0.5% permethrin) was chosen as the chemical pesticide to be tested due to its relatively low concentration making it safe to work with, though it still accurately represented a chemical that had been found in drinking water in the past (Permethrin in Drinking-Water, n.d.). The 20 μg/L concentration was estimated based on WHO guidelines for chemicals in drinking water, as this is the upper limit of the concentration of permethrin that is deemed acceptable (Syafrudin et al., 2021). While the insect repellant used in this study was far from pure permethrin, this guideline was used anyway due to the expected potential for additives to have similarly harmful effects. Little research was available on neem oil specifically at the time of this study, so for the purposes of this experiment, equal concentrations of permethrin and neem oil were used for their respective groups.

Stock solutions of the aforementioned chemicals were prepared shortly after the *D. rerio* hatched. To do this, 20 μL of Sawyer Permethrin Insect Repellent were added to a beaker containing 100 ml of distilled water in order to make a 1 mg/L stock solution. The use of a micropipette was necessary to dispense such a small quantity of liquid. This solution was then mixed thoroughly with a glass stirring rod. After doing so, 60 μL of the 1 mg/L stock were added to a 90 mm petri dish containing ~3 mL of 28.5°C aged aquarium water in order to create a concentration of 20 μg/L Insect Repellant. For the purposes of this study, all 'aged aquarium water' that was used also contained 10% methylene blue, an antibacterial agent included for the sake of reducing error and preventing outside contaminants from affecting the results.
The same procedure was then repeated for a second petri dish, this time using neem oil instead of the synthetic pesticide. However, although stirring the solution caused the neem oil to partially dissolve, it did not fully mix into the distilled water. The procedure was carried out anyway, but if the experiment were to be performed again, it may be ideal to incorporate an emulsifier into the solution in order to ensure the complete dissolution of the neem oil into the water. Finally, the last petri dish was filled with ~3 mL of aged aquarium water that was left untreated in order to serve as the environment for the control group.

To begin this study, 45 fertilized *D. rerio* eggs at roughly the same stage in development were obtained and kept in one large petri dish, this one containing the same fluid as the control group to avoid the larvae being harmed before hatching. At any given time throughout the experiment, all dishes containing *D. rerio* were kept in an incubator at 28.5°C as consistently as possible in order to facilitate their growth and development, including the period before the eggs had hatched (Singleman & Holtzman, 2014).

The petri dish was checked daily to see whether or not the eggs had all hatched. Once they had, *D. rerio* were moved via transfer pipette to the 90 mm petri dishes containing the solutions described previously, such that roughly 15 fish were present in each group. For the first two days after the *D. rerio* hatched, data was not collected due to the fact that their movements were very few and far between, and would have been difficult to draw conclusions from.

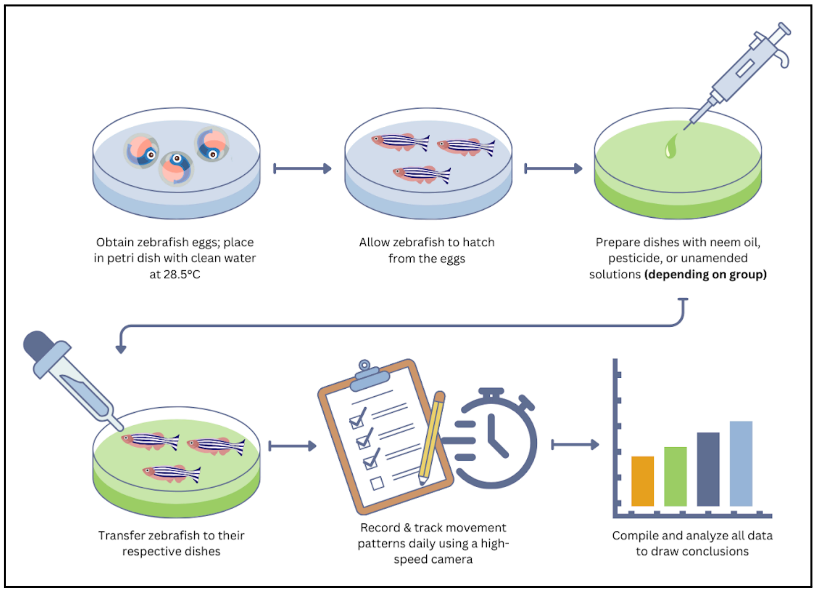


Figure 2. A simplified visualization of the methods used in this paper.

Due to their young age, *D. rerio* were not fed during the course of the experiment to avoid ammonia buildup, as changing the water would have been tedious and could have potentially skewed the results. *D. rerio* were able to subsist without food for the brief duration of the experiment, since they typically do not need to be fed until they're above the age of 7 days post-fertilization (Avdesh et al., 2012).

When the fish were at an age of 5 dpf, the data collection portion of the experiment began. Petri dishes were positioned underneath a high-speed camera (ordered from Fastec Imaging) one at a time such that the camera's field of view encompassed the entire petri dish, but little beyond that. This allowed for the closest possible view of the *D. rerio* while still ensuring that no movement would go unnoticed, or move outside of the camera's range. A flat, clear metric ruler was positioned underneath the petri dish in order to facilitate the process of measuring distance traveled, though it may have been ideal to move this ruler elsewhere in the frame or else to have ensured that the lines were grey as opposed to black. Since *D. rerio* appeared black on a grey background in the videos, the presence of the ruler lines below the clear dish meant that, when tracking *D. rerio* towards the end of the experiment, the fish would sometimes swim above the ruler lines and be difficult to see.

Once the petri dish was properly positioned, a ~5 second long video was recorded and saved for use in data analysis at a later date. This process was repeated for each group on the first day of data collection (5 dpf) and on two subsequent days, thus allowing the progression of any damage to the nervous system to be analyzed over the course of three days. On the 8th day post-fertilization, the experiment concluded, and all of the surviving *D. rerio* were removed from their containers and transferred to a healthy environment.

In order to measure the distance traveled by the *D. rerio* in each group, it was necessary to find a software that could allow their movements to be tracked based on the recorded videos. Numerous different programs were tested, including ZebraZoom and ANY-maze, but ultimately it was found that Fiji (ImageJ) was the most straightforward and practical to use for the purposes of this particular study (Mirat et al., 2013).

The only drawback to using this software was that it required movements to be tracked by manually clicking on the fishes' positions in each frame, which made the process more tedious than if it were to be automated. Additionally, this fact made the high frame rate of the camera used somewhat impractical, and footage had to be broken down so that *D. rerio* movements were plotted every 35 frames in order to make the procedure feasible. This didn't significantly limit the study, though, and once each fish in each group had been tracked, the gathered data was graphed and used to draw conclusions on the effects of chemical and natural pesticides on *D. rerio* movements.

Results

While mortality rate was not specifically tracked in this study, it was notable that all of the *D. rerio* in the natural pesticide group had died by the second day of tracking (6 dpf). On the first day (5 dpf), however, the fish in this group exhibited strange behaviors and appeared deformed to some extent. Photos of the fish were taken under a microscope in order to take note of their unusual appearances, as shown in Figures 3a and 3b, despite this not being the primary focus of the study.

Figures 3a and 3b. *D. rerio* from the control group (left) vs. the neem oil group (right) on the first day of tracking viewed under a microscope.

Tracking the *D. rerio* in Fiji provided data on the distance traveled by each fish, which was then compiled and displayed in numerous ways in order to make the data more digestible and allow conclusions to be drawn. Additionally, screenshots of the paths taken by some of the fish in each group were recorded in order to demonstrate the differences between the groups' movement patterns. The raw numerical data is not listed here due to the sheer amount of data gathered, which was a result of the distance traveled being measured over each of 86 frames for numerous different fish.

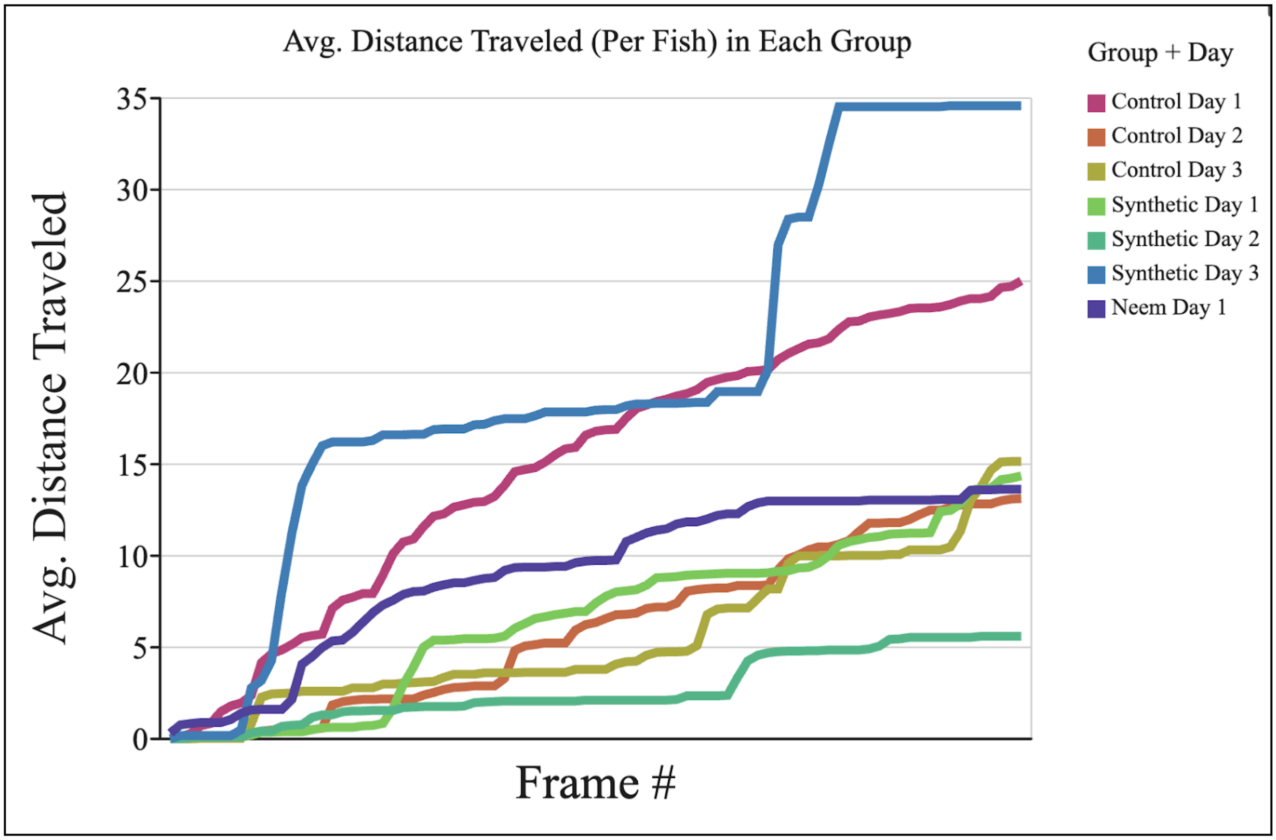


Figure 4 (above). Average distance traveled (in mm) over time for each group; each frame equates to ~60 ms.

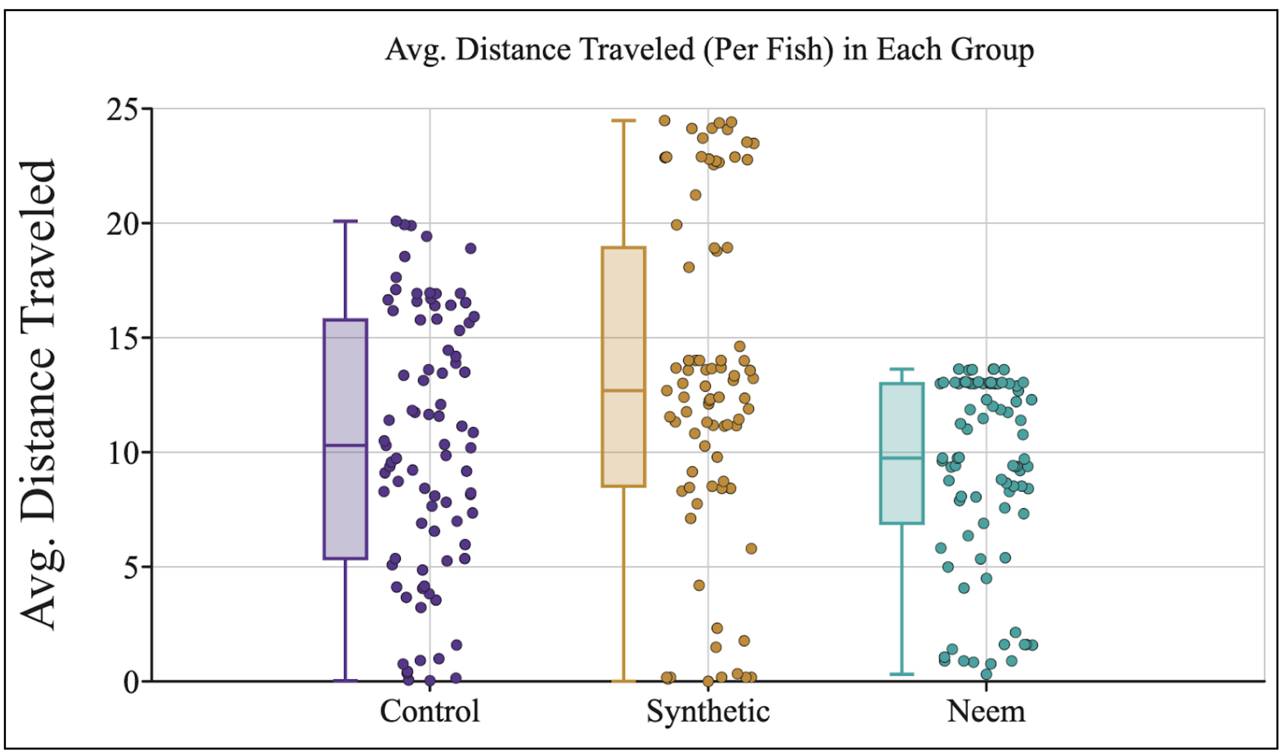
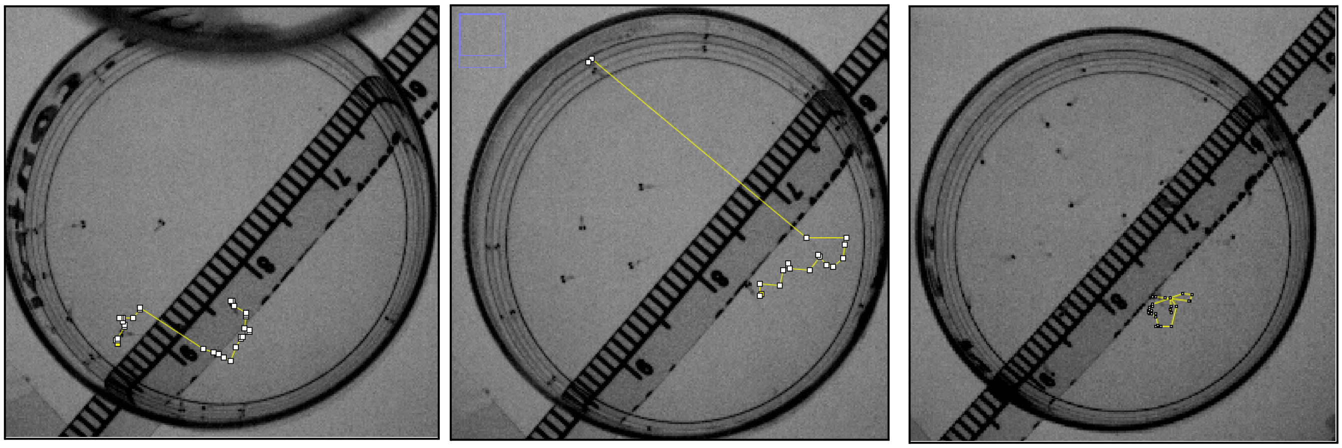


Figure 5 (above). Comparison of average distances traveled (in mm) per fish in each treatment group.



Figures 6a (left), 6b (middle), 6c (right). Tracked movement patterns of a fish in the control group (6a), the synthetic pesticide group (6b), and the neem oil group (6c) via Fiji.

Discussion

Ultimately, the data gathered in this study refuted the initial hypothesis, but provided valuable insight into the effects that synthetic pesticides and their alternatives had on *D. rerio* nervous systems. To begin, the control group behaved as expected for the duration of the experiment, a fact that can be seen clearly in each of the figures above. The fish in this group moved smoothly and did not appear to have been influenced by any outside factors, thus providing proof of the study's accuracy. The lack of unexpected results from the control group suggested that the differences between the groups were caused solely by the independent variable (i.e. the presence of a pesticide).

Figure 5 demonstrates the average cumulative distances traveled by fish in each individual group, essentially showing the amount of movement exhibited by *D. rerio* over time. These values were calculated by averaging together the distance traveled by each fish in a given group at a specific frame, with the time between each frame equating to ~3.5 seconds. As previously mentioned, increased or decreased locomotor activity can signify neurotoxic responses in *D. rerio*, and by looking at Figure 5, it can be clearly seen that the synthetic pesticide group moved a much larger distance on average than the others (d'Amora & Giordani, 2018). Statistical analysis performed on the data further confirmed this, with a p-value below 0.01 being calculated and thus indicating a strong confidence in the difference between the control and synthetic pesticide groups. Due to this confidence in the data, it was determined that synthetic pesticides had a significant negative impact on *D. rerio* nervous systems. Although the original hypothesis expected movement to decrease in response to the presence of pesticides, the drastic difference means that the ultimate implications of the data regarding neurotoxic damage still line up with preliminary research.

When looking at the plot of the neem oil group's movement in Figure 5, it can be seen that they appeared to travel a similar distance to the control group; the p-value was calculated to be roughly 0.32, implying a low confidence that any discrepancies were significant. This was likely due, in part, to the fact that there were fewer points of data for the neem oil group. Since this group had all died by the second day of testing, concrete comparisons could not be drawn using the same data as was used for the synthetic pesticide group.

While Figure 4 was not used for the purpose of performing statistical analysis, it serves as a useful reference for visualizing the data gathered throughout the duration of this experiment. The graph shows the average cumulative distance traveled by *D. rerio* in each group on each day, thus providing a deeper level of insight into how movement patterns differed over the course of the experiment, as well as how smoothly or erratically the fish moved. Figure 4 best demonstrates the abrupt nature of the movements exhibited by fish in the synthetic pesticide group, particularly on day 3 of the experiment, during which *D. rerio* primarily moved in short bursts and then slowed considerably.

Perhaps the most unexpected aspect of the results, however, was the severe harm that neem oil clearly caused to *D. rerio*. As previously stated, the fish in this group had all died before reaching 6 days post-fertilization, a mere 3 days after they were transferred to the petri dish containing the neem oil solution. While the neem oil group did not travel a significantly greater or lesser distance on average than the control group, as can be seen in Figures 4 and 5 (discussed previously), major differences were apparent in both the appearance and behavior of *D. rerio* in this group. Figures 6a, 6b, and 6c show the movement patterns of a single fish in each group. When comparing the neem oil group (6c) to the control group (6a), it can be seen that the fish in the former displayed an apparent inability to swim in a straight line. Instead, its movements were primarily circular, contrasting the relatively linear motion of the fish in the control group. This discrepancy indicates that *D. rerio* were severely harmed as a result of the neem oil's presence, albeit in a very different way from those in the synthetic group.

When considering this fact alongside the physical differences between *D. rerio* in these two groups, as demonstrated in Figures 3a and 3b, it can be seen that the neem oil caused a curvature in the fishes' bodies that appeared to be linked to their circular patterns of motion. Previous studies have suggested that body axis curvature in *D. rerio*, similar to that which was observed in this experiment, is directly linked to pyrethroid toxicity (DeMicco et al., 2009). Pyrethroids are commonly found in residential and agricultural insecticides; however, interestingly, neem oil does not contain any. This suggests that some other component of neem oil is responsible for its harmful effects–a point that future research may seek to explore. Notably, the group containing the synthetic pesticide solution was not visibly different from the control group, nor did any fish in the group die throughout the course of the experiment.

Being that the fish in the neem oil group were harmed to such an extent, the findings of this study suggest that runoff from natural pesticides may be equally as harmful to humans and aquatic life, if not even worse, than their synthetic counterparts. While the presence of chemical pesticides did indeed alter the activity levels of the fish, it did not cause any fatal damage, at least not for the duration of the study or at the tested concentration; these results refuted the initial hypothesis that synthetic pesticide exposure would cause a decrease in movement and that neem oil would have a lesser impact. Instead, the results of this study suggest that neem oil as a natural pesticide may not be an ideal alternative to synthetic pesticides due to the risk of it causing serious damage to aquatic ecosystems and, potentially, humans.

Limitations

Despite the intriguing results that this study unveiled, there were several areas in which it was limited to a certain degree. For one, *D. rerio* could no longer be used for the experiment once they were beyond 7 days post-fertilization in order to comply with ethical guidelines. This meant that the neurotoxic effects of natural and synthetic pesticides could not be examined over any extended period of time, leading this study to only expose short-term impacts. If future studies were to cover a longer duration, different or more in-depth results as to the effects of various pesticides on *D. rerio* locomotion could be gleaned.

The relatively small sample size used, that being ~15 fish in each group, contributed to the experiment's restrictions as well. Since *D. rerio* had to be tracked entirely by hand, any larger sample size was impractical, particularly given the time constraints of the study. While automated tracking systems were investigated as a possible solution to this, these did not ultimately work out, as those that were tested (e.g. ZebraZoom & AnyMaze) had issues with pinpointing the exact location of the *D. rerio* due to their clear bodies. Only their eyes were particularly easy to distinguish from the grey background, but the lines on the clear ruler that was used to ensure accurate measurements meant that the programs were unable to reliably keep track of each individual fish. This could be mitigated in the future by considering this drawback beforehand and taking efforts to ensure that *D. rerio* are clearly visible by some means, or by developing a new program specifically designed for the purposes of the study.

The lack of data for the neem oil group due to the fatalities following the first day of testing also hindered the accuracy of the results, as shown by the high p-value of 0.32 that was mentioned formerly. Though results could still be inferred from the mortality rate, curvature, and behavior of *D. rerio* in this group, it would be ideal for future studies to be able to better pinpoint the exact neurotoxic effects that neem oil had. In part, it is suspected that some of the aforementioned harm caused by the neem oil may have been influenced by the fact that it did not dissolve fully when combined with water, making the substance added to the petri dish containing the natural pesticide group more of a mixture than a solution. While it is not certain whether or not this fact caused any significant difference in the study's findings, it could be worthwhile for future research to attempt these methods again while combining an emulsifier with the neem oil solution.

Future Directions

While the initial purpose of this study–that is, to find a safer alternative to traditional synthetic pesticides–was ultimately left unachieved, these findings have the potential to pave the way for future research in the area that might finally be able to solve the increasingly pressing issue of pesticide pollution. There are a number of ways in which the methods used in this paper could be expanded upon. For one, only a single, constant concentration of both types of pesticides was tested; however, in reality, the concentration of pesticides in water varies greatly across the globe (Navarro et al., 2024). In many cases, pesticide concentrations in drinking water may even end up being significantly lower than the 20 μg/L that were used in these methods. Future research could build upon that which was presented in this study by testing the effects of multiple concentrations of natural and synthetic pesticides on *D. rerio*. Not only would this contribute to the larger body of knowledge by design, but using a smaller concentration may lead to a longer lifespan for the neem oil group, thus allowing the effects of the natural pesticide to be analyzed in greater detail over a longer period of time.

In order to extend the duration of this study, the potential for different model organisms to be used in place of *D. rerio* could also be explored. Using an invertebrate could be ideal, as these typically come with fewer ethical regulations–the primary drawback to the use of *D. rerio*, as explained previously, was the fact that they can only be used in an experiment for a limited amount of time. The nature of the invertebrates should be researched as well, however, in order to ensure that the model organism chosen is not a pest that would typically be intended as a target for these pesticides, since this could affect the results and their ability to be applied to humans.

Finally, although it may be among the most potent natural pesticides, neem oil is far from the only alternative to synthetic pesticides that could be tested. For instance, diatomaceous earth (DE) is another naturally-occurring insecticide that some theorize to be healthier for the environment, and as it comes in the form of a powder as opposed to an oil, it could be found to have different impacts on *D. rerio*. Where neem oil works by suffocating insects or impacting their hormone function in order to prevent reproduction, DE instead causes insects to dry out, leading to their deaths. Since this insecticide works best when kept dry, it's possible that it may cause far less harm to aquatic life–and to humans who drink water containing it–than neem oil.

References

Adnan, S. M., Uddin, M. M., Alam, M. J., Islam, M. S., Kashem, M. A., Rafii, M. Y., & Latif, M. A. (2014). Management of Mango Hopper, *Idioscopus clypealis,* Using Chemical Insecticides and Neem Oil. *The Scientific World Journal*, *2014*, 1–5. https://doi.org/10.1155/2014/709614

Aishwarya Garud, Pawar, S., Patil, M. S., Kale, S. R., & Patil, S. (2024). A Scientific Review of Pesticides: Classification, Toxicity, Health Effects, Sustainability, and Environmental Impact. *Cureus*. https://doi.org/10.7759/cureus.67945

Alarcon, W. A., Calvert, G. M., Blondell, J. M., Mehler, L. N., Sievert, J., Propeck, M., Tibbetts, D. S., Becker, A., Lackovic, M., Soileau, S. B., Das, R., Beckman, J., Male, D. P., Thomsen, C. L., & Stanbury, M. (2005). Acute Illnesses Associated With Pesticide Exposure at Schools. *JAMA*, *294*(4), 455–455. https://doi.org/10.1001/jama.294.4.455

Avdesh Avdesh, Chen, M., Martin-Iverson, M. T., Mondal, A., Ong, D., Rainey-Smith, S., Taddei, K., Lardelli, M., Groth, D. M., Verdile, G., & Martins, R. N. (2012). Regular Care and Maintenance of a Zebrafish (Danio rerio) Laboratory: An Introduction. *Journal of Visualized Experiments*, *69*. https://doi.org/10.3791/4196

Bjørling-Poulsen, M., Andersen, H. R., & Grandjean, P. (2008). Potential developmental neurotoxicity of pesticides used in Europe. *Environmental Health*, *7*(1). https://doi.org/10.1186/1476-069x-7-50

Burtscher-Schaden, H., Durstberger, T., & Zaller, J. (2022). Toxicological Comparison of Pesticide Active Substances Approved for Conventional vs. Organic Agriculture in Europe. *Toxics*, *10*(12), 753–753. https://doi.org/10.3390/toxics10120753

d'Amora, M., & Giordani, S. (2018). The Utility of Zebrafish as a Model for Screening Developmental Neurotoxicity. *Frontiers in Neuroscience*, *12*. https://doi.org/10.3389/fnins.2018.00976

DeMicco, A., Cooper, K. R., Richardson, J. R., & White, L. A. (2009). Developmental Neurotoxicity of Pyrethroid Insecticides in Zebrafish Embryos. *Toxicological Sciences*, *113*(1), 177–186. https://doi.org/10.1093/toxsci/kfp258

Doszyn, O., Dulski, T., & Zmorzynska, J. (2024). Diving into the zebrafish brain: exploring neuroscience frontiers with genetic tools, imaging techniques, and behavioral insights. *Frontiers in Molecular Neuroscience*, *17*. https://doi.org/10.3389/fnmol.2024.1358844

El Afandi, G., & Irfan, M. (2024). Pesticides Risk Assessment Review: Status, Modeling Approaches, and Future Perspectives. *Agronomy*, *14*(10), 2299–2299. https://doi.org/10.3390/agronomy14102299

How zebrafish can help the science of neurodegeneration. (2018, April 5). Uq.edu.au. https://qbi.uq.edu.au/blog/2018/04/how-zebrafish-can-help-science-neurodegeneration

Michael. (2009). Introduction: Pesticides Use and Exposure, Extensive Worldwide. *Reviews on Environmental Health*, *24*(4). https://doi.org/10.1515/reveh.2009.24.4.303

Mirat, O., Sternberg, J. R., Severi, K. E., & Wyart, C. (2013). ZebraZoom: an automated program for high-throughput behavioral analysis and categorization. *Frontiers in Neural Circuits*, *7*. https://doi.org/10.3389/fncir.2013.00107

Muhammad Syafrudin, Risky Ayu Kristanti, Adhi Yuniarto, Hadibarata, T., Rhee, J., Al-onazi, W. A., Tahani Saad Algarni, Almarri, A. H., & Al-Mohaimeed, A. M. (2021). Pesticides in Drinking Water—A Review. *International Journal of Environmental Research and Public Health*, *18*(2), 468–468. https://doi.org/10.3390/ijerph18020468

Navarro, I., de, A., Sanz, P., Abrantes, N., Campos, I., Alaoui, A., Christ, F., Alcon, F., Contreras, J., Glavan, M., Igor Pasković, Marija Polić Pasković, Trine Nørgaard, Mandrioli, D., Sgargi, D., Hofman, J., Aparicio, V., Baldi, I., Bureau, M., & Vested, A. (2024). Assessing pesticide residues occurrence and risks in water systems: A Pan-European and Argentina perspective. *Water Research*, *254*, 121419–121419. https://doi.org/10.1016/j.watres.2024.121419

Nougadère, A., Sirot, V., Kadar, A., Fastier, A., Truchot, E., Vergnet, C., Hommet, F., Baylé, J., Gros, P., & Leblanc, J.-C. (2012). Total diet study on pesticide residues in France: Levels in food as consumed and chronic dietary risk to consumers. *Environment International*, *45*, 135–150. https://doi.org/10.1016/j.envint.2012.02.001

Permethrin in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality. (n.d.). https://www.who.int/docs/default-source/wash-documents/wash-chemicals/2004-permethrin-background.pdf

Saputra, F., Lai, Y.-H., Marri, Alos, H. C., Aventurado, C. A., Vasquez, R. D., & Hsiao, C.-D. (2023). The Effect of the Pyrethroid Pesticide Fenpropathrin on the Cardiac Performance of Zebrafish and the Potential Mechanism of Toxicity. *Biology*, *12*(9), 1214–1214. https://doi.org/10.3390/biology12091214

Singleman, C., & Holtzman, N. G. (2014). Growth and Maturation in the Zebrafish,*Danio Rerio*: A Staging Tool for Teaching and Research. *Zebrafish*, *11*(4), 396–406. https://doi.org/10.1089/zeb.2014.0976

Tai, C., Horzmann, K. A., Franco, J., Jannasch, A. S., Cooper, B. R., & Freeman, J. L. (2021). Developmental atrazine exposure in zebrafish produces the same major metabolites as mammals along with altered behavioral outcomes. *Neurotoxicology and Teratology*, *85*, 106971–106971. https://doi.org/10.1016/j.ntt.2021.106971

What is a Pesticide? | US EPA. (2014, October 3). US EPA. https://www.epa.gov/minimum-risk-pesticides/what-pesticide

Widrick, J. J., Lambert, M. R., Kunkel, L. M., & Beggs, A. H. (2023). Optimizing assays of zebrafish larvae swimming performance for drug discovery. *Expert Opinion on Drug Discovery*, *18*(6), 629–641. https://doi.org/10.1080/17460441.2023.2211802