Clove Essential Oil Inhibitory Properties on Acetylcholinesterase

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INTRODUCTION

Insecticides have been used for many years to prevent agricultural pests from preventing diseases and destroying crops. There are various types of insecticides that target specific pests. One type of insecticide is malaoxon, which is an organophosphate insecticide that targets a range of pests, including bean beetles (*Callosobruchus maculatus*). Insecticides work by inhibiting the enzyme acetylcholinesterase (AChE) which disrupts the insect's nervous system, causing paralysis and death (Araújo et al., 2023). AChE is an enzyme in the nervous system that terminates nerve impulses by catalyzing the hydrolysis of acetylcholine (ACh) (Araújo et al., 2023). However, chemical insecticides such as malaoxon can be hazardous to humans, other organisms and the environment (Jensen & Whatling, 2010). Thus, companies are searching for innovative ways to protect crops against pests while minimizing the adverse effects caused by chemical insecticides.

One of these different strategies to replace chemicals such as malaoxon is the use of natural insecticides. Currently, Dr. Firooznia and his colleague J. Morillo are studying the use of plant essential oils as natural insecticides to control pests, such as the bean beetle. Essential oils are secondary metabolites; plant-produced compounds that are not directly involved in growth or reproduction (West, 2024). There is a wide variety of essential oils and many have strong antibacterial properties (West, 2024). Additionally, they are environmentally friendly and easily accessible (West, 2024).

Clove oil is a type of essential oil derived from clove trees (*Syzygium aromaticum*) (Bai et al., 2023). The primary component of clove oil is eugenol which can inhibit certain enzymes vital for the metabolic processes of microbes, however, it is unclear if clove oil inhibits enzymes required for insect functioning (Bai et al., 2023). Research has been conducted on the pesticidal properties of clove oil. A study by Hamza & Hamza (2018) determined that clove oil, when mixed with lambda-cyhalothrin and a low concentration of alpha-cypermethin is useful in protecting wheat grains against the lesser grain borer (*Rhyzopertha dominica*). Using clove oil reduced the amount of other chemical insecticides required when it showed that clove oil may reduce environmental pollution, lower risks to human health and delay insect resistance development (Hamza & Hamza, 2018). Another study showed that clove oil and its constituents have been determined to cause a significant population reduction of the pear psyllid (*Cacopsylla chinensis*)when applied to infected pear trees (Topal, 2019). Ultimately, this study demonstrated that clove oil and its constituents have the potential as a source of natural insecticide (Topal, 2019).

While some research has explored the pesticidal properties of clove oil, there is no clear answer that tells us if clove oil is effective in inhibiting acetylcholinesterase and is a powerful insecticide. This study aims to investigate how effective clove oil is at inhibiting acetylcholinesterase and to compare its effects to the chemical insecticide malaoxon, which has been proven to be effective in pest control but poses significant risks to human health and the environment. We hypothesize that clove oil will effectively inhibit the enzyme acetylcholinesterase based on the extensive research that has determined clove oil's antimicrobial and potential pesticide properties.

METHODS

The methods for this experiment are exactly as stated in BIOL3510 Lab Manual, Topic 3 The essential oil we used was clove oil. A two tailed t-test was used to compare the difference between the malaoxon and methanol treatments and to compare the difference between the clove oil and methanol treatments. It is also important to note that for the results of absorbance, the blank absorption was subtracted from all treatment readings to account for any background absorbance.

RESULTS

There were differences in absorbance among the three treatments used in our enzyme assay. Between the three treatments, the lowest mean absorbance at 412 nm was clove oil, with a mean absorbance of 0.0346 between the five clove oil tubes.   The five malaoxon-treated tubes (positive control) had the second lowest mean absorbance at 0.0538, and the 5 methanol-treated tubes (negative control) had the highest mean absorbance at 2.0324. Figure 1 visually represents how the absorbance at 412 nm compares between the different treatments.

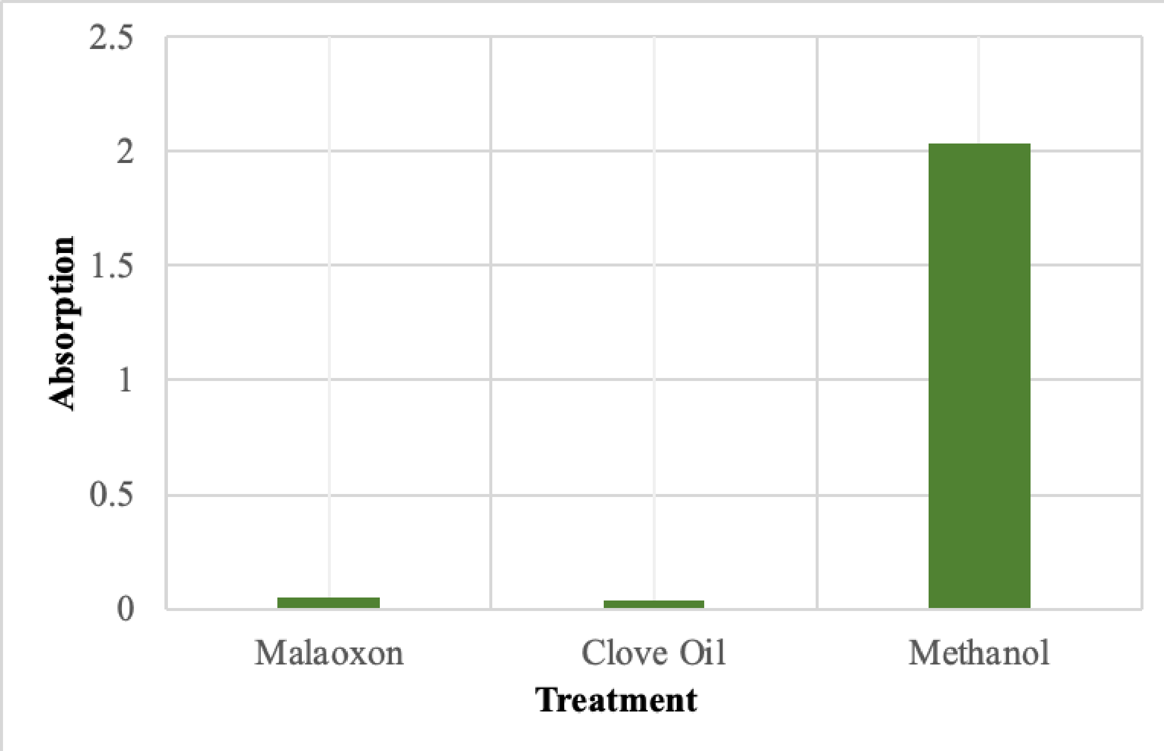

Figure 1. Absorption of various treatments at 412 nm of light after procedure.

A t-test between malaoxon and methanol showed a p-value of 9.74 x10^-6, and a t-test between clove oil and methanol resulted in a p-value of 5.46 x10^-7. Both p-values are significantly below 0.05, which demonstrates that both malaoxon and clove oil have significantly lower absorbances than the negative control methanol.

DISCUSSION

The mean absorbance values showed a difference in absorbance at 412 nm of light between the different treatments. Methanol had the highest absorbance, with an average value of 2.0324, which was significantly higher than the naloxone and clove oil treatments. This suggests that AChE was functioning normally in the presence of methanol, the negative control, which resulted in high absorbance. This is because when DTNB (DiThiobisNitroBenzoate) is added to purified AChE, it forms a yellow dianion, when AChE is not inhibited, that can be detected by the spectrophotometer and therefore would result in more light absorbed at 412 nm.

There was a significant difference in absorbance between methanol and malaoxon (p-value = 9.74 x 10^-6) and between methanol and clove oil (p-value = 5.46 x 10^-7), as the p-values were below 0.05. This indicates that AChE inhibition occurred in both the malaoxon and clove oil treatments because less amounts of the yellow dianion would result in a much lower absorbance value when compared to our negative control treatment methanol. Because the clove oil-treated tubes had a significantly lower average absorption when compared to our negative control treatment, methanol, we can confidently reject the null hypothesis. Further, the significant differences between the negative control (methanol) and the positive control (malaoxon) suggest that both malaoxon and clove oil inhibited the AChE enzyme.

However, It was surprising that clove oil had a lower absorbance than malaoxon, even though malaoxon has been provenoften to be a quality insecticide by inhibiting AChE. Although the main focus of our study was not to compare malaoxonand clove oil (the main focus of our study was to see if clove oil has AChE inhibitory properties), it would be interesting to do further research and see if clove oil is a better inhibitor of AChE than malaoxon. However, one thing that stood out,was the higher absorbance from our clove oil blank tube (0.482) when compared to the absorbance from the malaoxonblank tube (0.148). Perhaps there may have been some error in measuring the absorbance of the clove oil blank tube which could have led to the lower mean absorbance values in comparison to malaoxon.

This study aligns with other studies that showed clove oil's ability to reduce populations of specific pests, such as the pear psyllid or the lesser grain borer, by demonstrating that clove oil causes a decrease in these pests, perhaps because of its ability to inhibit AChE (Topal, 2019). Other studies suggest that clove oil has enzyme-inhibition properties in microbes (Bai et al., 2023). Because there is evidence of enzyme inhibition by clove oil, it further strengthens the notion that it could inhibit the enzyme AChE.

Overall, this study suggests that clove oil could serve as an effective insecticide due to its ability to inhibit the AChE enzyme.While further research, including studies in natural environments, is necessary to assess the full impact of clove oil as an insecticide, its potential offers a promising alternative to the harmful effects of many chemical insecticides currently in use. In conclusion, clove oil and other essential oils could represent the future of natural insecticides, helping to minimize the negative environmental and health impacts associated with conventional insecticides.

References

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Topic Questions:

1. It is important to add the substrate after the insecticide or plant essential oil during the enzyme assay because if the substrate is added before, the reaction between AChE and the substrate will have already occurred and thus produced acetic acid and thiocholine which interact with DTNB. Adding the substrate defeats the purpose of "inhibiting" the function of AChE because the function would have already occurred.
2. The type of t-test appropriate for this analysis is a two-tailed t-test. This is because we have two independent groups (i.e. methanol and clove oil) and are determining if their means are significantly different from one another.
3. Yes, it is possible for plant essential oils to be harmful/toxic to humans and to ecological systems. For example, it was mentioned in a recent study that plant essential oils may pose aquatic organisms at risk (Afonso et al., 2024). Essential oils can cause irritation to the skin and if ingested can cause irritation to the stomach and digestive tract. Further, inhaling certain essential oils can worsen asthma symptoms in sensitive individuals. Strong essential oils such as orange and clove may irritate the airways and cause bronchoconstriction, coughing, or difficulty breathing. It is important to note that some essential oils may cause more irritation than others and thus I would suggest that it is not completely safe to use these products without assessing their safety. If, for example, someone does have asthma and thinks that the smell of the orange essential oil may be beneficial to have in their room, but they do not look at how the oil can cause respiratory irritation due to its strong scent, they may suffer from respiratory irritation.

https://www.lung.org/blog/essential-oils-harmful-or-helpful#:~:text=Inhalation%20of%20certain%20essential%20oils,coughing%2C%20or%20shortness%20of%20breath.

https://doi.org/10.1016/j.scitotenv.2023.168467

4. Yes, clove oil can serve as a natural remedy for improving human health. One key study that I read about by Bai et al. (2018) delved deep into the antimicrobial properties of clove oil. The researchers found that because of the eugenol content in clove oil, it has a wide range of antibacterial effects that make it effective against foodborne pathogens such as E. coli and S. aureus. The mechanism by which this works begins with eugenol being derived from clove oil. The eugenol then damages the cell wall and membrane, causing the cellular contents to leak out of the cell and ultimately resulting in cell death. Additionally, the study proposes other mechanisms, such as the inhibition of biofilm formation, the oxidative stress-mediated apoptosis (which is essentially targeted lysis of the bacteria) and the disruption of DNA synthesis.

Bai et al. LWT 173:114249.

5. Yes, I strongly believe, based on this study, that our understanding of plant secondary metabolites can be used to foster sustainable agriculture, enhance pest management strategies and enhance crop breeding to enhance resilience. More research into the molecular mechanisms behind the production of these metabolites can lead to even more effective and targeted solutions for sustainable agriculture in the future.