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## Investigating the Effectiveness of Natural Biopesticides: Testing Essential Oils and Wormwood Extract on Hazelnut and Asian Bed Bugs Vs Acetamiprid

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**Figure 1: Bedbugs in Petri Dish**



**Figure 2: Essential Oil Droplet Applied to the Bed Bug's Metathorax**

### Abstract

Bed bugs, both native and invasive species, present a growing threat to agricultural crops and human health. The newly introduced Asian bed bug, particularly concerning due to its resistance to common insecticides, further complicates pest control efforts. This study explores the potential of essential oils applied directly to the bed bug's metathorax as a natural control method. While promising, direct application in agriculture raises concerns about potential harm to crops, applicator safety, and cost, despite their prevalent use in food preparation. Initial experiments exploring less expensive application methods produced less encouraging results.

Beyond efficacy, this study delves deeper into safety considerations. Using computational tools (Flare Cresset), researchers compared the effects of essential oil components and synthetic active ingredients on both human

and midge acetylcholinesterase, a key enzyme in the nervous system. This comparison aims to assess potential risks associated with essential oil use.

Overall, this research investigates the potential of essential oils for bed bug control while acknowledging crucial safety considerations and economic feasibility for agricultural application. Further research is needed to optimize delivery methods, address cost concerns, and ensure safety for humans and beneficial insects.

**Keywords:** Natural Products, Insecticide, Pest Management and Computational Chemistry

## Introduction

Bedbugs (*Cimicidae* spp.) pose a significant threat to both agricultural crops and human health [1]. Their recent emergence in Western countries, particularly the Asian bed bug, presents a unique challenge due to their resistance to common insecticides. While reintroducing "suspended molecules" offers an alternative, their use remains restricted due to toxicity concerns. (University of Arizona; Arizona Pest Management Center).

This study investigates the effectiveness of essential oils applied directly one drop to the insect's metathorax as a potential bed bug control method. It compares the effectiveness of these oils with computational models of their individual components and synthetic active ingredients, focusing on their inhibitory effect on acetylcholinesterase, a common target for insecticides.

Several essential oils from the Lamiaceae family, including oregano (terpineol-type), peppermint, rosemary, and sage, exhibit anti-acetylcholinesterase activity. Notably, oregano (phenol-type) even inhibits both acetylcholinesterase and butyrylcholinesterase. Additionally, pretreatment with lavender oil, linalool, or linalyl acetate offers protection against scopolamine-induced toxicity, potentially due to reduced intracellular ROS levels in linalool. Furthermore, these essential oils, except lavender, demonstrated neuritogenic activity at 100 µg/mL, with oregano (terpineol-type) showing the strongest effect [2].

Investigated the potential of essential oils as an alternative to control bed bugs. Here's what they found, Promising candidates, Among 15 tested components, carvacrol and thymol were most effective, though less potent than conventional insecticides. Impact on nervous system: Carvacrol, thymol, and eugenol inhibited the bed bug nervous system, potentially disrupting vital functions. Linalool, however, had the opposite effect, stimulating the nervous system [3].

**New insights for Control:** These findings can guide the development of effective essential oil-based insecticides and shed light on how they work.

**Limitations:** While promising, essential oils were less potent than conventional insecticides. Further research is needed to address issues like short product life and odor concerns. In this study it is carried out docking simulations on wormwood extract compounds, highlighting the potential efficacy of essential oil on human acetylcholinesterase confirmed by [4].

## Materials and Methods

### Bed Bug's Test Execution Mode

The insects are taken from the fixtures in the same day, inserted in a petri dish together with a slice of golden apple and then treated applying directly to the insect's metathorax. By carrying out tests on invertebrates in this case of the *Cimicidae* family, the opinion of the Committee for Experiments on Animals is not required (European Commission).

### Insects Used for Treatment

1 bed bug *Cimex lectularius*  
7 Asian bedbugs *Halyomorpha halys*  
31 bedbugs of the *palomena prasina* hazelnut  
divided into 14 petri dishes

### Treatments Used in the Test (See Supplementary Material Preparations):

- Wormwood (*Artemisia absintium*) hydroalcoholic extract
- Essential oils of rosemary (*Salvia rosmarinus*), sage (*Salvia officinalis*), thyme, (*Thymus vulgaris*) mountain pine (*Pinus mugo*) applied as such 1 drop per insect
- Acetamiprid and water dilutions

### Essential Oils Dilutions in

- tween 20 (polysorbate)
- extra virgin olive oil

- acetone
- alcohol

## Computational Tools

Computational calculations were performed by comparing the scores obtained by docking compounds present in essential oils in the binding pocket of fruit fly acetylcholinesterase. The compounds of rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*) and thyme (*Thimus vulgaris*) were downloaded in SDF format from PubChem. See reference. See supplementary tables 1,2,3.

- Docking sage essential oil compounds inside the acetylcholinesterase binding pocket on fruit fly acetylcholinesterase (ProteinDataBank ID 6ARY). See reference.
- Comparison between the binding pocket of human acetylcholinesterase (PDB ID 6NEA) and fruit fly acetylcholinesterase (PDB ID 6ARY)

## Results

### Bed Bug Test Results

#### Application Test of Acetamiprid Compared with Wormwood Extract

The dilution of acetamiprid indicated on the label killed 100% of the insects like absinthe extract applied as is. By diluting acetamiprid and wormwood extract with water, the percentage number of dead insects decreased as indicated in table 1

Sample no	1	2	3	4	5	6	7	8	9	10	11	12	13	14
type of treatment	A In Tal	E as it is	A 1/2	E 1/2	A 1/3	E 1/3	A 1/5	E 1/5	A 1/7	E 1/7	A 1/10	E 1/10	A THYME	C
	*	*	*	*	*		*				*			
% dead insects	100%	100	50	100	25	75	0%	50%	0%	25%	0%	0%	0%	0%

\*= ASIAN BED BUG  
 So, fourteen groups with an Asian bed bug in seven  
 C= control= mains water  
 A= wormwood, A1/2= diluted wormwood extracts 50% A(1/3. 1/10)  
 E= Acetamiprid as in label E(1/3. 1/10)

**Table 1: Indication of Group Number, Type of Treatment and Dilution**

### Undiluted Essential Oils Test Result Application

The treated bedbugs died almost immediately with the essential oil of rosemary and sage, 2 videos uploaded on YOUTUBE (visible only to those who have the link).

<https://youtu.be/u3w5M-hhf1A> [https://youtu.be/\\_i\\_TJmQlkk](https://youtu.be/_i_TJmQlkk)

- With thyme essential oil insects died in about 1/2 hour.
- With the essential oil of mountain pine (test on 2 insects) one died almost instantly the other in a few minutes.

### Results of the Application of Essential Oils Diluted with Tween 20

the dilution significantly reduced the effectiveness of the essential oils as shown in table 2.

polysorbate dilutions	1/2	1/3	1/5	1/7	1/10	1/15	1/20	1/30	1/40	1/70	1/100
		**		**	**	**					
%ID	100%	75%	50%	0%	0%	0%	25%	25%	0%	0%	0%

\*\* = asian bedbugs.  
 %ID: % dead insects.

**Table 2: Dilution of Rosemary Essential Oil with Tween (Polysorbate) 20 Test Results**

### Dilution with Extra Virgin Olive Oil Rosemary Essential Oil

After the application of the drop of solution there is a reaction comparable to convulsions for vertebrates, and then a slow recovery in 15-20 hours.

### Sage Essential Oils Diluted with Acetone

In the test carried out by diluting the essential oil of sage with acetone it significantly reduced its effectiveness as indicated in table 3, the acetone applied as is had no effect.

acetone dilutions	1/2	acetone	1/5	1/7	1/10	1/15	1/20	1/30	1/40	1/70	1/100
	M	V	V**	M	V**	V**	V	V	V	M	V
%ID											

Only 1 bed bug per dilution  
V\*\*=After the application of the drop of solution there is a reaction comparable to convulsions for vertebrates, and then a slow recovery in 15-20 hours  
V= alive M= dead

**Table 3: Sage Essential Oils Diluted with Acetone**

### Sage Essential Oils Diluted with Alcohol

In the test carried out by diluting the essential oil of sage with alcohol it significantly reduced its effectiveness as indicated in table 3, the alcohol applied as is had no effect.

Alcohol dilution	1/2	alcohol	1/5	1/7	1/10	1/15	1/20	1/30	1/40	1/70	1/100
	M	V	V**	V**	V	V	V	V	V	M	V

Only 1 bed bug per dilution.  
V\*\*=After the application of the drop of solution there is a reaction comparable to convulsions for vertebrates, and then a slow recovery in 15-20 hours.  
V= alive M= dead.

**Table 4: Sage Essential Oils Diluted with Alcohol**

### Computational Analysis Result

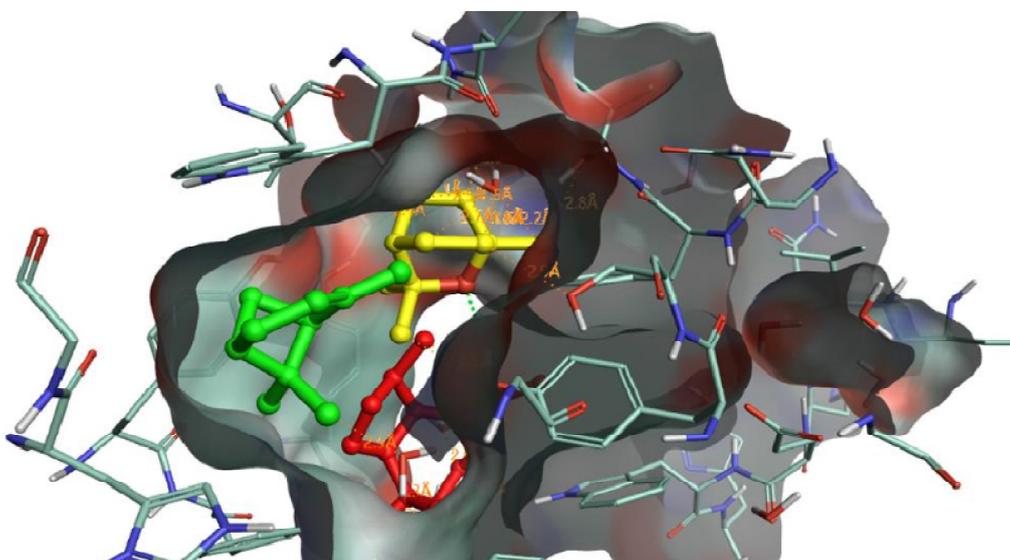
#### Docking Sage Essential Oil Compounds Inside the Acetylcholinesterase Binding Pocket

Among the compounds identified in the essential oils of rosemary, sage and thyme (Tables 1, 2, and 3), viridiflor, eucalyptol and alpha-pinene achieved the highest scores in indicate the specific evaluation or analysis. See Figure 4 for details [2].

#### See Screenshot 1 In Additional Data

Docking simulations suggest that multiple sage essential oil compounds can simultaneously occupy the acetylcholinesterase binding pocket, potentially leading to synergistic inhibition of the receptor. See fig. 4 This finding aligns with research by Perry et al [4]. who suggest that the insecticidal activity of essential oils primarily stems from their main terpenoid constituents acting as acetylcholinesterase inhibitors. Additionally, they propose a significant synergistic effect among these constituents, as long as no single compound is potent enough to solely explain the inhibitory action [5].

Given that terpenoids are low-molecular-weight molecules, they have the potential to bind simultaneously within the binding pocket. Therefore, further in vitro testing of individual compounds in combination (2 or 3) could be valuable to evaluate how such synergy occurs.

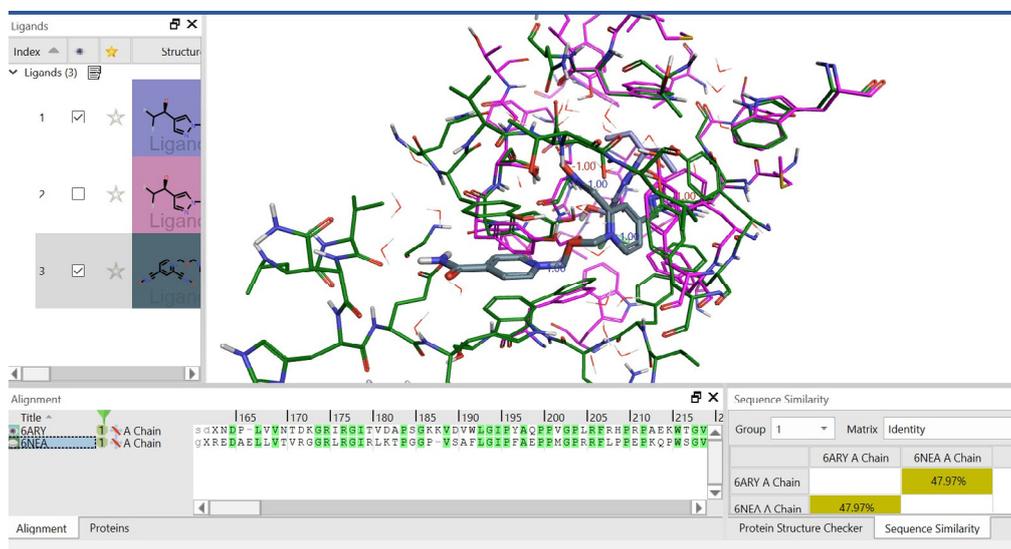


**Figure 3: Sage Essential Oil Compounds Simultaneously Inside the Acetylcholinesterase Binding Pocket in Viridiflor Red, Eucalyptol Yellow and Alpha Pinene Green, With the Surface of the Binding Pocket Highlighted**

## Computational Comparison Between the Binding Pocket of Human Acetylcholinesterase and Fruit Fly

A computational comparison of the binding pockets in human and fruit fly acetylcholinesterase reveals a high degree of similarity, with an alignment and overlap score of 47.9% (Figure 5). This suggests potential challenges in developing insecticides that specifically target insect acetylcholinesterase without affecting the human enzyme.

Insecticides like acetamiprid exploit this structural similarity, demonstrating higher affinity for insect acetylcholinesterase compared to the human version. This highlights the need for further research into more selective targeting mechanisms to minimize potential risks to human health. See figure 4.



**Figure 4: Alignment and Overlap of a Chain of Human Acetylcholinesterase (Pdb Id 6nea in Green) and Fruit Fly (Pdb Id 6ary In Fuchsia) Similar to 47.9% of the Binding Site**

### Discussion

While this study demonstrates the potential of essential oils as a natural bed bug control method, several key points warrant further discussion.

#### Efficacy and Selectivity

- The direct application method proved highly effective, with some essential oils causing rapid death. However, diluted applications and alternative application methods require further investigation to optimize their effectiveness while balancing cost and practicality.
- The selective toxicity observed with wormwood extract suggests potential for developing species-specific control strategies with minimal impact on non-target beneficial insects.

#### Cost and Feasibility

- The high cost of commercially available essential oils presents a significant barrier to their widespread adoption in agriculture. Exploring farm-produced options and utilizing aromatic waters offer promising cost-effective alternatives.
- The potential impact of essential oils on the organoleptic properties of food requires further investigation, particularly for agricultural applications where food quality is paramount.

#### Mechanism of Action and Safety

- The computer simulations provide valuable insights into the potential mechanism of essential oils, suggesting their possible inhibition of the acetylcholinesterase enzyme. Further research with individual terpenoids can elucidate the synergistic effects observed.
- The potential human and environmental safety of both essential oils and potential breakdown products must be thoroughly assessed before practical implementation.

#### Future Directions

- Optimizing delivery methods and evaluating the efficacy of different application techniques are crucial for widespread adoption.
- Investigating the synergistic effects of specific terpenoid combinations within essential oils can shed light on their potential as safer and more effective alternatives to traditional insecticides.
- Conducting comprehensive safety assessments of essential oils and their byproducts is essential for ensuring their safe application in agricultural and other settings.

## Conclusions

This study evaluated the effectiveness of essential oils (rosemary, sage, thyme, mountain pine) and a hydroalcoholic wormwood extract against various bed bug species (green bed bug, hazel bed bug, Asian bed bug). Acetamiprid positive control, Synthetic insecticide, and water (negative control) served as comparisons. Direct application of essential oils to the insect metathorax proved highly effective, causing death in some cases almost instantly. Videos demonstrate these results (links provided).

The wormwood extract exhibited selective toxicity, proving lethal to the common bed bug but only causing mild numbness in other species at high concentrations. The synthetic insecticide achieved complete lethality at the recommended dosage and partial lethality at a diluted concentration. Diluting essential oils with olive oil, acetone, or ethyl alcohol significantly reduced their toxicity. While 50% dilution led to rapid death, lower dilutions resulted in slower recovery from initial "convulsive" phases.

Despite their effectiveness, the high retail cost of essential oils (around €1/gram) poses a significant barrier to their use.

The study suggests exploring farm-produced essential oils and utilizing aromatic waters (dilute residues containing 1% essential oils) as potential cost-effective and residue-free alternatives. However, the potential impact of essential oils on the organoleptic properties of food (taste and aroma) remains an unknown factor requiring further investigation. Computer simulations show that compounds within sage essential oil can fit together inside the acetylcholinesterase binding pocket, potentially weakening the enzyme's function in a combined way (synergy).

This supports previous research suggesting that terpenoids in essential oils act as natural acetylcholinesterase inhibitors. Since these terpenoids are small molecules, they could potentially bind together within the pocket. Further testing of individual terpenoid combinations in lab experiments could help understand how this synergy works [2].

In conclusion, this study presents encouraging evidence for the potential of essential oils as a natural bed bug control method. However, addressing the limitations and concerns discussed necessitates further research to ensure their cost-effectiveness, environmental safety, and compatibility with agricultural practices. By combining these findings with further investigation, we can move closer to implementing sustainable and effective methods for bed bug control.

## Founding

The study project is self-financed

## Acknowledgements

I gratefully acknowledge Cresset for providing me with an academic license, which enabled the computational calculations and visualizations of proteins and molecules presented in this study.

## Artificial Intelligence

Part of the text has had its grammar corrected using artificial intelligence <https://gemini.google.com/>

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### Supplementary material

See supplementary material

### Video Links

#### Video presentation of the project:

[https://www.canva.com/design/DAF7IegxU6A/2L6mXb2iuHaI91Z4CPaTfQ/watch?utm\\_content=DAF7IegxU6A&utm\\_campaign=designshare&utm\\_medium=link&utm\\_source=editor](https://www.canva.com/design/DAF7IegxU6A/2L6mXb2iuHaI91Z4CPaTfQ/watch?utm_content=DAF7IegxU6A&utm_campaign=designshare&utm_medium=link&utm_source=editor)

#### Video Test

<https://youtu.be/u3w5M-hhf1A>

[https://youtu.be/\\_i\\_TJmQl0kk](https://youtu.be/_i_TJmQl0kk)