N-ReoC

Salvia rosmarinus and *Cocos nucifera* Leaf Combined Essential Oils as A Novel, Eco-friendly, and Cost-effective Nanopesticide Against *Tribolium castaneum*

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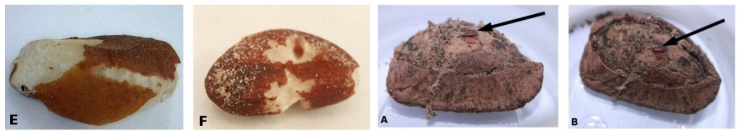
INTRODUCTION & PROBLEM STATEMENTS

- *Tribolium castaneum* is a **cosmopolitan** **major stored grain pest** with **high reproductive rate** due to the presence of **4,8-dimethyldecanal in air**, an aggregation pheromone.[1]
- *T. castaneum* invade through small cracks and gaps in package seals and cause damage by feeding on stored grain products and contaminating them with cast skins, dead insects, and faeces, causing pungent odours
- Their presence is also linked to accelerated development of the mycotoxigenic fungus *Aspergillus flavus* which produces **Aflatoxin B1**,[2] **genotoxic hepatocarcinogen** that leads to **serious liver-related diseases** such as **hepatocellular carcinoma (liver cancer)**
- This leads to a significant loss of **stored grain quality** and **quantity** with **serious economic loss**, resulting in **$220B USD loss per annum** in stored grain products. This leads to more than **800 million people** not having access to a **safe food supply** and indirectly causing **famine**. (FAO)
- *T. castaneum* causes post-harvest grain loss at **40%** and cereal loss at **30%** in India alone annually. (FAO)
- Their **small size**, **short life cycle** and **easy maintenance** make *T. castaneum* **ideal for laboratory studies.**[2]
- *T. castaneum* has also been shown to develop **metabolic resistance** to **almost all synthetic pesticides**.[~4]
- These reasons, combined with the significant damage caused, led us to choose *T. castaneum* as the **testing pest model.**
- Studies have shown that **nanopesticides** can potentially act as an effective solution for the current **overuse of synthetic pesticides**.
- O/W (oil-in-water) nanoemulsions with plant-based essential oils can mitigate these impacts as a safer and more effective substitution as they exhibit **higher efficacy** against the targeted pest while having **longer lasting effect** and **little to no cytotoxicity**.
- The formulation of O/W nanopesticides with multiple essential oils has yet to be researched by October 2023, resulting in **untapped benefits**.
- **Decreased risk** of development of **metabolic resistance** due to the complex mixture of bioactive compounds.

Tribolium castaneum (Coleoptera: Tenebrionidae) (Peggy Greb, 2016)



Damages caused by the Tribolium castaneum adults to intact Brazil nuts 90 days (E and F) of exposure. // T. castaneum in a Brazil nut with a cracked shell, after 30 days of infestation. (A and B) (E. M. Pires et al., 2017)

WHY NOT SYNTHETIC PESTICIDES, PLANT-BASED ESSENTIAL OILS, REPELLENT, PHOSPHINE GAS???

Synthetic Pesticides

- Highly **cytotoxic**
- **Leaves residue in food and water** which lead to **cell mutation** and other serious health issues when consumed
- There are more than **110,000–168,000 deaths** globally each year contributed by pesticide poisoning[3]
- Causes **ecological imbalance** when non-targeted organisms affected by pesticide poisoning

- *Coleoptera* has been shown to develop **1000-fold resistance** rapidly when exposed to synthetic pesticide, as previous studies proven that *T. castaneum* has developed metabolic resistance to almost **all synthetic insecticides**[4]

Plant based EOs

- **Not cytotoxic**
- **Does not cause insecticidal resistance**
- However it **loses its effectiveness within short period** due to its **poor physico -chemical properties** (water insolubility, high volatility and quick half-life)
- *T. castaneum* usually appear in granaries which are closed and packed environments
- This makes EOs **more difficult** to apply onto stored products

Repellent

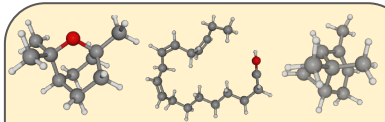
- Not suitable for stored products
- Stored grain is stored in closed and packed environments
- If repellent is applied, pests will simply move to another grain sack and cause **secondary damage** to another grain sack without solving the main problem

Phosphine (PH_3) Treatment

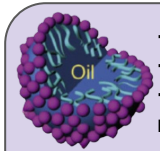
- Highly **cytotoxic** with 100% mortality rate
- Reports have shown that the widespread use of phosphine heavily contributed towards building up high resistance levels (**119-fold more resistant**) in *T. castaneum* with **resistance frequencies of 94%**[5]
- Phosphine inhibits the human body's ability to produce proteins and subsequently result in severe cellular, tissue, and organ damage and, ultimately, **death**. (CDC)

Why rosemary EO, coconut leaf EO and O/W nanoemulsion?

- Abundant and perennial
- Non-seasonal plants
- Easily accessible
- High yield of essential oil



- Both EO's major bioactive compounds possess insecticidal properties with high abundance
- Non-ecotoxic



- Cheap
- Kinetically stable
- Enables control release system
- improve the poor physico-chemical properties of both EOs

We propose **rosemary and coconut leaf combined essential oils** with **O/W nanoemulsion** as a **nanopesticide** against *T. castaneum* (N-ReoC) as the solution. As of October 2023, there has been **NO RESEARCH** conducted on **Rosemary and Coconut Leaf Combined Essential Oils** nor **Combined Essential Oils** with **O/W nanoemulsion** as **nanopesticide** against *T. castaneum.*

RESEARCH QUESTIONS

-Is the nanoemulsion-based combination of the *Salvia rosmarinus* and *Cocos nucifera* leaf essential oils (N-ReoC) a **novel, eco-friendly, and cost-effective nanopesticide** against *Tribolium castaneum*?

-What is the relationship between **concentration** and the **mortality rate** on *T. castaneum* of N-ReoC?

–How do the **individual concentrations** of nanoemulsion-based *Salvia rosmarinus* and *Cocos nucifera* leaf essential oils and the nanoemulsion-based **combination of both essential oils** (N-ReoC) affect the **mortality rate** of *T. castaneum*?

-Does **nanoemulsion-based N-ReoC** have a **higher mortality rate** compared to **non-nanoemulsion-based N-ReoC** at the **same concentrations**?

OBJECTIVES

-To determine whether the nanoemulsion-based combination of Salvia rosmarinus and Cocos nucifera leaf essential oils is a **novel, eco-friendly, and cost-effective nanopesticide** against T. castaneum.

-To determine the relationship between the **concentrations of N-ReoC** and the **mortality rate** of N-ReoC against T. castaneum.

-To determine the **relationship** between the **individual concentrations** of nanoemulsion-based Salvia rosmarinus and Cocos nucifera leaf essential oil and the **nanoemulsion-based combination of both essential oils** (N-ReoC) and the mortality rate of T. castaneum.

-To determine whether **nanoemulsion-based N-ReoC** has a **higher mortality rate** compared to **non-nanoemulsion based N-ReoC** at the **same concentrations**.

HYPOTHESES

-N-ReoC is a **novel, eco-friendly and cost-effective nanopesticide** against T. castaneum.

-The higher the **concentration of N-ReoC**, the higher the **mortality rate** of T. castaneum.

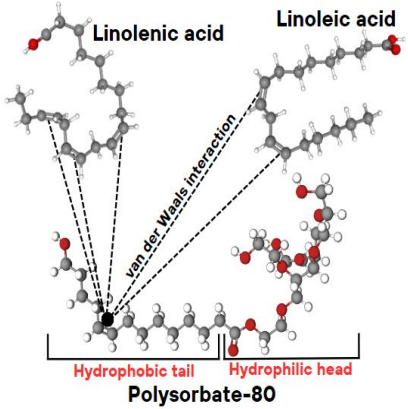
-**Nanoemulsion-based N-ReoC** has a **higher mortality rate** against T. castaneum compared to the **individual nanoemulsion-based essential oils** of Salvia rosmarinus and Cocos nucifera leaf.

-**Nanoemulsion-based N-ReoC** has a **higher mortality rate** compared with **non-nanoemulsion-based N-ReoC** at the **same concentrations**.

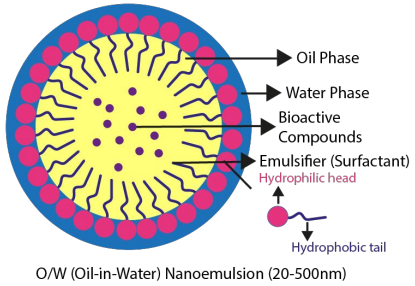
SCIENTIFIC CONCEPT

Nanoemulsions

- Emulsions in which the droplet size is **between 20 – 500 nm.**
- In N-ReoC, Polysorbate-80 acts as a **surfactant monolayer** that encapsulates the bioactive compounds of rosemary and coconut leaf EOs.
- Polysorbate-80 having **Hydrophilic–lipophilic balance value (HLB) of 15** and the **presence of an unsaturated bond at aliphatic chain** causes the forming of **van der Waals interactions** with the **unsaturated bonds** of **linolenic acid** and **linoleic acid**.
- Polysorbate-80 **adsorbed effectively** around the oil droplets and **reduced the surface tension** at interfacial layer by forming a **surfactant monolayer**.
- This resulted in **smaller particle size, lower zeta potential,** a **surfactant monolayer** that prevent **ostwald ripening**, **aggregation** and **degradation** from happening and it also enables **passive controlled release system** with **long lasting effect** that releases **active ingredients over time**, thus improving its **physico-chemical properties**
- Due to the subcellular droplet size of the emulsions, N-ReoC is able to **directly adsorb** onto the surface of *T. castaneum* and **penetrate through the exoskeleton** and cell membrane, which causes **oxidative stress** and **denaturation of organelles and enzymes**, resulting in **cell death**.



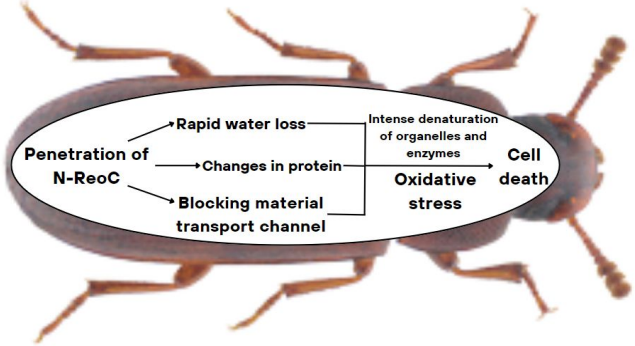
van der Waals interaction of polysorbate-80 with linolenic acid and linoleic acid



O/W (oil-in-water) nanoemulsion with polysorbate-80 as a surfactant monolayer for Rosemary and Coconut leaf EOs

Penetration enhancing effect of 1,8-cineole

- The cuticle/exoskeleton of *T. castaneum* is considered a **biphasic structure,** consisting of a **waxy hydrophobic/lipophilic outer layer**.
- It has a **large surface area**, making it the most important route of insecticides exhibiting contact toxicity into an insect's internal physiology.
- 1,8-cineole lowers the surface tension of the wax layer, **increasing the affinity and spreadability** of other bioactive compounds such as camphor.
- Since the efficacy of an insecticide is directly dependent on its ability to penetrate the cuticle and bind to its target sites, this **improves the synergy** between the complex mixture of bioactive compounds of Rosemary EO and **improves the mortality** of N-ReoC.

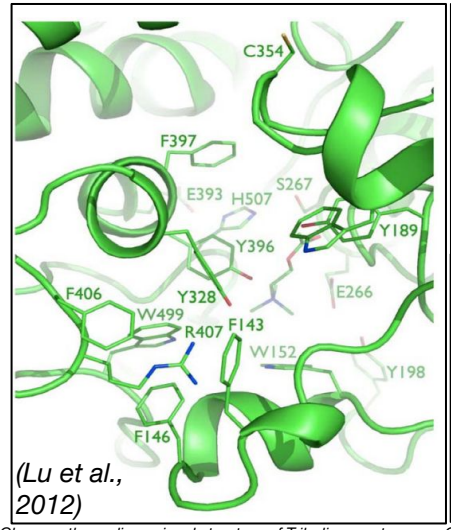


Mode of Action of Nanoparticles of N-ReoC against T.castaneum

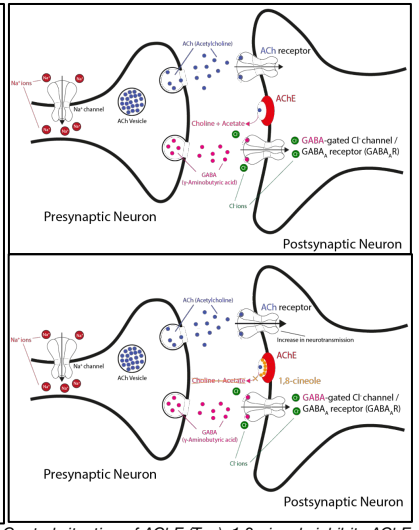
SCIENTIFIC CONCEPT

Inhibition of Acetylcholinesterase (AChE)

- **1,8-cineole** and **α-Pinene,** major constituents of Rosemary essential oil (EO), act as **inhibitors of acetylcholinesterase (AChE**)
- These compounds **competitively inhibit acetylcholinesterase** by binding to the **catalytic triad** as well as the **choline binding site** of AChE via **π-alkyl interactions** with the indole ring of W152.
- Covalent catalysis of acetylcholine (ACh) is also prevented by the binding to 1,8-cineole and α-Pinene to residues E199 & H507 in the catalytic triad of acetylcholinesterase.
- 1,8-cineole and α-Pinene also bind to 8 and 7 residues respectively in the peripheral anionic site (PAS) to form a **reversible enzyme-substrate complex** through **Van der Waals forces**, **π-alkyl interactions** and **π-sigma interactions**, preventing ACh from binding to AChE
- ACh accumulates in the cholinergic sites and continuously stimulates the cholinergic nerve fibres, leading to constant neurotransmission and neuronal hyperexcitation, and subsequently, paralysis and **insect death**



Close up three-dimensional structure of Tribolium castaneum TcAce1/AChE1 (Lu et al., 2012)



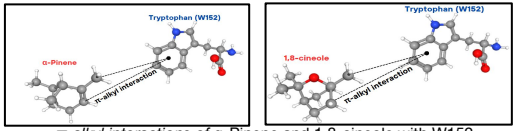
Control situation of AChE (Top); 1,8-cineole inhibits AChE, preventing acetylcholine (ACh) from binding to AChE(Bottom)

Antagonism of 5-HT

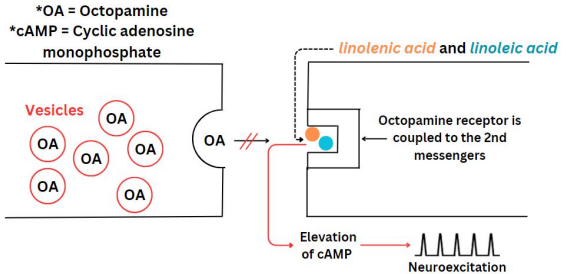
- 5-hydroxytryptamine (5-HT) or serotonin controls the **modulation of heart rate**, **nutrition signalling** and **aggression** in insects, including *T. castaneum*
- 1,8-cineole stimulates the **5-HT$_7$** receptors in *T. castaneum*, **increasing intracellular cyclic adenosine monophosphate (cAMP)** levels (Schlenstedt et al., 2006) as well as inhibiting the AChE of T. castaneum, causing **mortality**

Agonism of Octopamine

- Octopamine (OA) is a biogenic monoamine that functions as a neurohormone, neuromodulator and neurotransmitter, regulating various physiological functions in insects
- Linoleic acid, palmitic acid and linolenic acid act as **competitive OA agonists** and **bind to β-adrenergic-like octopamine receptors (OctβRs)**, causing the **activation of adenylate cyclase** and induces an **increase in intracellular cAMP concentration**
- These changes are associated with **neuronal hyperexcitation**, **tracheal blockage** and **membrane disruption**, and eventually, **insect death**



π-alkyl interactions of α-Pinene and 1,8-cineole with W152



Linolenic acid and linoleic acid acting as competitive octopamine (OA) receptor agonists

PREPARATION OF N-ReoC

Rosemary and coconut leaf distillates are extracted via Hydrodistillation

Liquid-liquid extraction is done with both distillates and n-hexane (10:1), aqueous phase is isolated

Organic phase is put into water bath machine (85°C) for evaporation to obtain essential oil (EO)

Both EOs are stirred with polysorbate-80 (1:1:4 ratio) and distilled water (50mL) for 15 minutes

Solution is ultrasonicated with water bath sonicator for 15 minutes

N-ReoC is obtained!!!

EXPERIMENTAL DESIGN

1. Labelling System

Concentration

Positive/Negative Control

$(NA/NN)X_n$ - Y g dm^{-3} X_n - Ctrl (N/P)

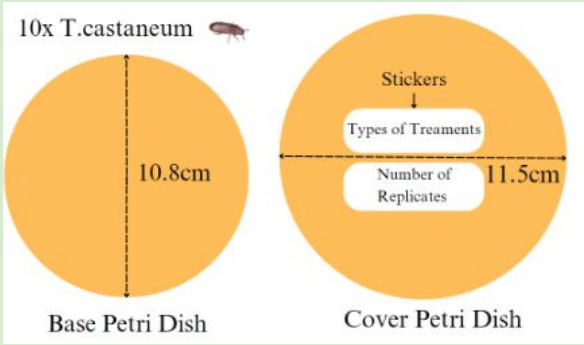
Nano/Non-nano
NA - nano
NN - non-nano

Type of treatment
R - Rosemary EO
C - Coconut leaf EO
CR - Rosemary and coconut leaf EO Combined

DWP - distilled water with polysorbate-80

Number of specimens
-10
BD - Bayer Deltacide

2. Contact Toxicity Bioassays

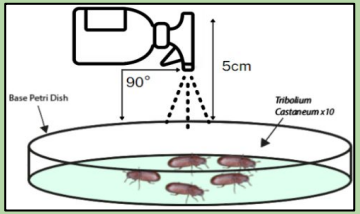


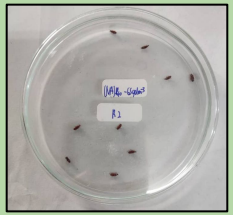
Replicates
-R1 / R2 /R3

* Base petri dish will be covered up with cover petri dish once the treatment is sprayed into the base petri dish

3. Synergistic Effect Tests

To determine the **synergistic effect** of the **combination of both essential oils** (N-ReoC) compared to its **individuals constituent** (all treatments with nanoemulsion applied)





Total Sprays:
3x (±0.2 mL)
Time intervals (180s per intervals):
180s, 360s, 540s, 720s, 900s, 1080s

Treatments:
- Nano Rosemary EO
- Nano Coconut Leaf EO
- Nano N-ReoC

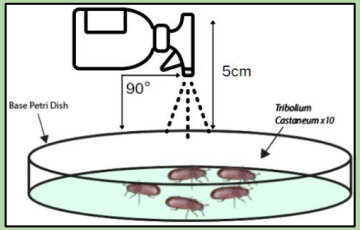
Concentration (5g dm^{-3} per interval):
40g dm^{-3}, 45g dm^{-3}, 50g dm^{-3},55g dm^{-3}, 60g dm^{-3} , 65g dm^{-3}

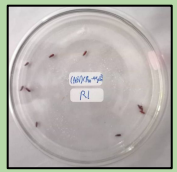
Negative Control:
50mL Distilled water with 10mL polysorbate-80

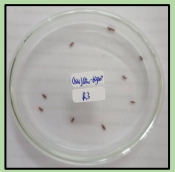
Positive Control:
Bayer Deltacide solution

4. Non-nano/nano Tests

To determine the **mortality difference** between **non-nanoemulsion-based N-ReoC** and **nanoemulsion-based N-ReoC** with the same testing concentration applied.







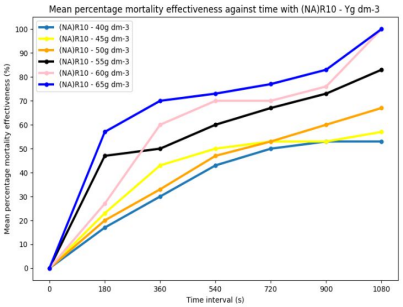
Total Sprays:
3x (±0.2 mL)
Time intervals (180s per intervals):
180s, 360s, 540s, 720s, 900s, 1080s

Treatments:
- Non-nano N-ReoC
- Nano N-ReoC

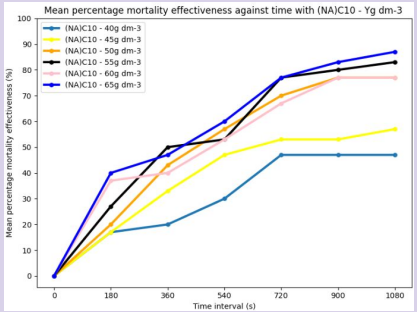
Concentration (5g dm^{-3} per interval):
40g dm^{-3}, 45g dm^{-3}, 50g dm^{-3}, 55g dm^{-3}, 60g dm^{-3} , 65g dm^{-3}

SYNERGISTIC EFFECT TESTS RESULT

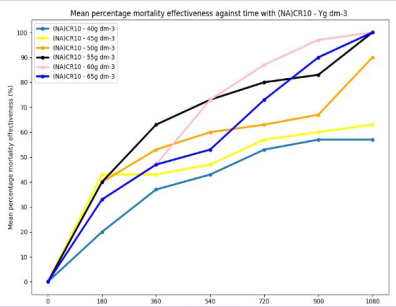
Nanoemulsion-based Rosemary EO



Nanoemulsion-based Coconut Leaf EO



Nanoemulsion-based N-ReoC

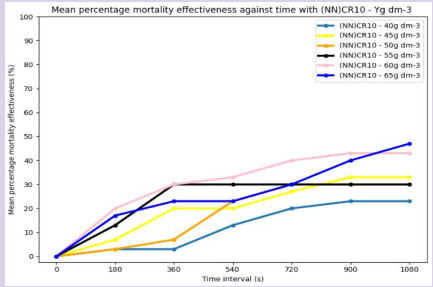


- At 1080 seconds, **Rosemary EO** achieved **100% mortality rate** starting from **60g dm^{-3}**
- At 1080 seconds, **Coconut EO** only reached **86.667 ± 3.333% mortality rate** with its highest testing concentration of **65g dm^{-3}**
- At 1080 seconds, **N-ReoC** achieved **100% mortality rate** starting from **55g dm^{-3}**

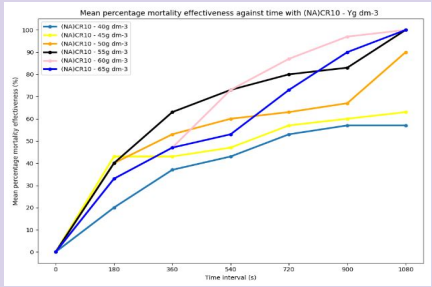
- **55g dm^{-3}** is the **optimum concentration** of **N-ReoC** as the higher concentration of **60g dm^{-3}** and **65g dm^{-3}** reached the **same mortality rate** at 1080 seconds

NON-NANO / NANO TESTS RESULT

Non-nanoemulsion-based N-ReoC



Nanoemulsion-based N-ReoC



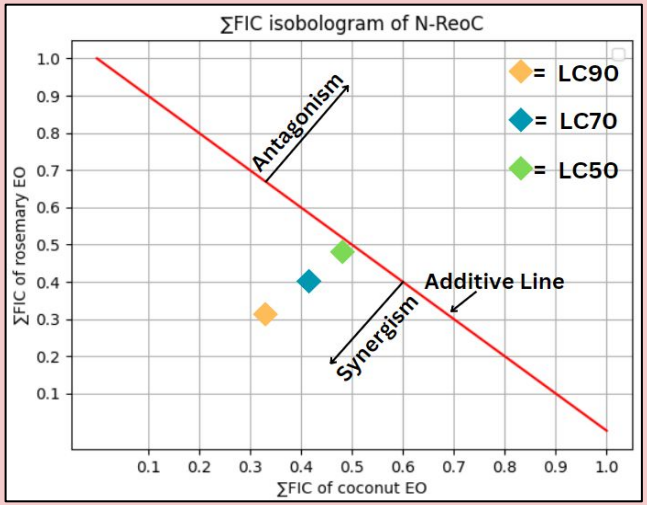
- At 1080 seconds, **Non-nanoemulsion-based N-ReoC** only achieved **47 ± 3.333% of mortality rate** with the highest testing concentration, **65g dm^{-3}**
- At 1080 seconds, **Nanoemulsion-based N-ReoC** achieved **100% mortality rate** starting from **55g dm^{-3} to 65 g dm^{-3}**

Lazar Modular Framework Acute Toxicity Prediction[6]

	1,8-cineole	α-Pinene	Camphor	Linolenic Acid	Linoleic Acid	Palmitic Acid
FM	75.8 mg/kg	50.77 mg/kg	49.8 mg/kg	314 mg/kg	3040 mg/kg	356 mg/kg
DM	n/a	n/a	n/a	38000 mg/kg	5790 mg/kg	12200 mg/kg

- Result shown that all major constituent in both EO with **low acute toxicity** on *Fathead minnow (FM)* and *Daphnia magna (DM)* as testing models

∑FIC ISOBOLOGRAM ANALYSIS



Lethal concentration (LC)	∑FIC value
LC_{50}	0.4860215
LC_{70}	0.4106644
LC_{90}	0.3232810

* Synergy = (∑FIC ≤ 0.5)

- ∑FIC formula is modified by replacing inhibitory concentration to lethal concentration to find out the **synergistic effect** between both essential oils.
- **∑FIC value gets lower** as the **concentration of N-ReoC increases.**
- The ∑FIC Index value of N-ReoC is **lower than 0.5**, confirming a **synergistic effect** between both essential oil with nanoemulsion applied (N-ReoC).

SYNERGISTIC EFFECT TESTS TWO-WAY ANOVA

Tests of Between-Subjects Effects

Dependent Variable: mortality

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	174.593[a]	17	10.270	34.662	<.001	.942
Intercept	3204.741	1	3204.741	10816.000	<.001	.997
concentration	145.926	5	29.185	98.500	<.001	.932
treatment	16.037	2	8.019	27.062	<.001	.601
concentration * treatment	12.630	10	1.263	4.263	<.001	.542
Error	10.667	36	.296			
Total	3390.000	54				
Corrected Total	185.259	53				

a. R Squared = .942 (Adjusted R Squared = .915)

- There is **statistically significant interaction** between treatment solution and concentration ($F(10, 36) = 4.263$, **$p = < .001$**).

NON-NANO/NANO TESTS TWO-WAY ANOVA

Tests of Between-Subjects Effects

Dependent Variable: Mortality

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	276.972[a]	11	25.179	41.202	<.001	.950
Intercept	1167.361	1	1167.361	1910.227	<.001	.988
Concentration	47.806	5	9.561	15.645	<.001	.765
Treatment	210.250	1	210.250	344.045	<.001	.935
Concentration * Treatment	18.917	5	3.783	6.191	<.001	.563
Error	14.667	24	.611			
Total	1459.000	36				
Corrected Total	291.639	35				

a. R Squared = .950 (Adjusted R Squared = .927)

- There is **statistically significant interaction** between treatment solution and concentration ($F(5, 24) = 6.191$, **$p = < .001$**).

POST-HOC: TUKEY'S HSD

Multiple Comparisons

Dependent Variable: mortality

Tukey HSD

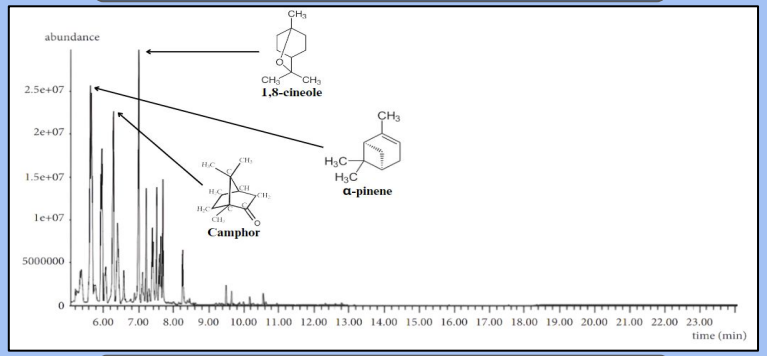
(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
rosemary	coconut leaf	.7222*	.18144	<.001	.2787	1.1657
	N-ReoC	-.7222*	.18144	<.001	-1.1657	-.2787
coconut leaf	rosemary	-.7222*	.18144	<.001	-1.1657	-.2787
	N-ReoC	-1.4444*	.18144	<.001	-1.8879	-1.0009
N-ReoC	rosemary	.7222*	.18144	<.001	.2787	1.1657
	coconut leaf	1.4444*	.18144	<.001	1.0009	1.8879

Based on observed means.
The error term is Mean Square(Error) = .296.
*. The mean difference is significant at the .05 level.

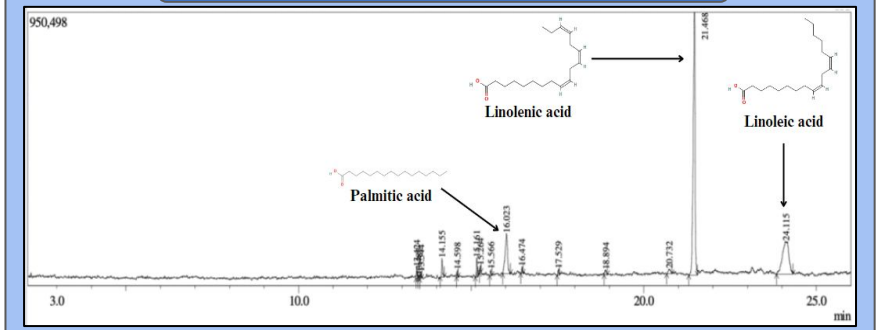
- The mortality percentage of N-ReoC is **statistically significantly different** from rosemary EO and coconut leaf EO, **(p = < .001)**.

GC-MS ANALYSES

Rosemary Essential Oil



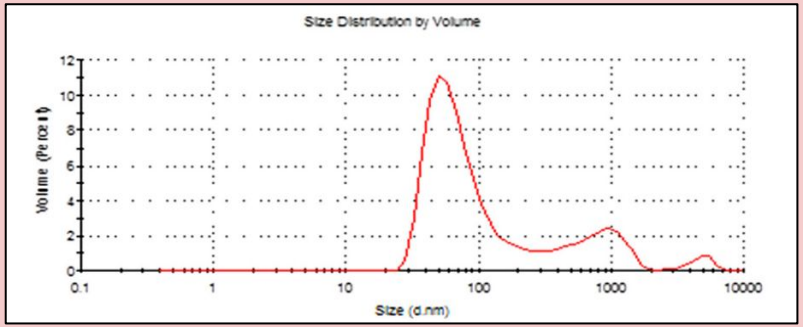
Coconut Leaf Essential Oil



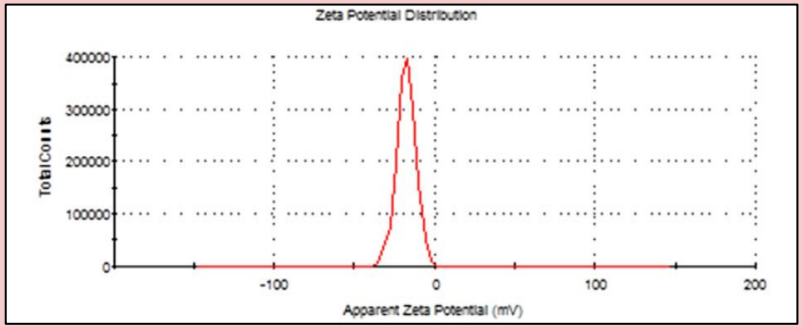
- Rosemary EO major compounds were **1,8-cineole** (32.18%), **α-Pinene** (15.4%) and **Camphor** (16.2%)
- Coconut Leaf EO major compounds were **linolenic acid** (55.84%), **linoleic acid** (23.74%) and **palmitic acid** (10.68%)

PARTICLE SIZE ANALYSES RESULTS

Size Distribution and PDI of N-ReoC



Zeta Potential of N-ReoC



- Size distribution graph of N-ReoC shown the average size of **83.41nm** (Peak 1) and average width of **60.46nm** (Peak 1) with PDI (polydispersity index) value of **0.457**
- The zeta potential distribution was shown to be **-17.72mV** and zeta deviation value was shown to be **5.86mV**

Discussion

1. Chemical properties analyses

Rosemary EO ← **GC-MS** → **Coconut leaf EO**

- **1,8-cineole** (32.18%)
- **camphor** (16.2%)
- **α-Pinene** (15.4%)

Sorted ascendingly based on the % value

- **linolenic acid** (55.84%)
- **linoleic acid** (23.74%)
- **palmitic acid** (10.68%)

Particle Size and PDI Value Analysis

Size (Peak 1)	Width (Peak 1)
83.41nm	60.46nm

PDI value 0.457

This indicates a **good uniformity in droplet size distribution,** where the **PDI value lower than 1 (0.457 < 1).**[7]

Zeta Potential Analysis

Zeta Potential (Peak 1) = -17.72mV

Zeta Deviation (Peak 1) = 5.86mV

Low zeta potential indicates that N-ReoC remains **kinetically stable for a long period of time**[7] (-30mV < **-17.72mV** < +30mV)

2. Statistical Analyses

LAZAR toxicity prediction with *Fathead minnow* and *Daphnia magna* showed **low acute toxicity** by bioactive compounds

ΣFIC value of N-ReoC is **0.323**, showing a **significant synergistic effect** between Rosemary and Coconut Leaf EO

The **LC_{50} difference** between nanoemulsion N-ReoC (**40.459g dm^{-3}**) and non-nanoemulsion N-ReoC (**85.700g dm^{-3}**) is **45.241g dm^{-3}**.

Tukey's HSD Test shown that the mean value of mortality percentage of N-ReoC **differs** from that of rosemary EO (**p = <.001**, 95% C.I. = (.2787, 1.1657)) and coconut leaf EO (**p = <.001**, 95% C.I. = (1.0009, .2787)).

Two-way ANOVA test for both tests showed a **statistically significant interaction** between treatment solution and concentration.
Synergistic effect test => (F (10, 36) = 4.263, **p = < .001**)
Non-nano/nano test => (F (5, 24) = 6.191, **p = < .001**)

3. Interpretation of Results

Concentration ↑
→ **% Effective collisions** ↑
→ **Binding rate** ↑
→ **Mortality rate** ↑

As the concentration of N-ReoC increases, the number of bioactive compounds per unit volume of N-RepC increases.
The percentage of effective collision between the bioactive compounds with their target sites increase, causing N-ReoC to have a **higher binding rate** as well as **mortality rate.**

N-ReoC induced **100% mortality** at **optimum concentration**: **55g dm^{-3}**
Higher concentrations (60, 65g dm^{-3}) are also able to attain 100% mortality but leads to unnecessary cost expenses.

Optimum concentration

- **No wasted material**
- **100% Mortality rate**

Subcellular droplet size of nanoemulsion + **Penetration enhancing effect of 1,8-cineole** =

Penetration of N-ReoC through *T. castaneum* exoskeleton is boosted → **Mortality rate of N-ReoC is improved significantly**

Positive control (BAYER Deltacide solution) induced **100% mortality**.
Negative control (Distilled water/Polysorbate-80 solution) showed **no mortality** towards *T. castaneum* specimens.
This proves that the mortality rate of the *T. castaneum* specimens was **not influenced by external factors**.

CONCLUSION

Hypotheses Are Accepted!!!

- ✅ **N-ReoC is a novel, eco-friendly and cost-effective nanopesticide.**
- ✅ **N-ReoC's mortality rate increases with the concentration (Optimum concentration = 55g dm^{-3}).**
- ✅ **N-ReoC's mortality rate is higher than rosemary EO and coconut leaf EO individually due to the synergistic effect of dual-pathway mechanism (ΣFIC value = 0.323, synergism = 0.323<0.5).**
- ✅ **N-ReoC's mortality rate is higher than non-nanoemulsion-based N-ReoC (Up to 53.333%).**

COST ANALYSIS

Type of treatment	Fipronil	N-ReoC	Malathion
Price per gram (USD g^{-1})	2000	0.109	0.10
Pollution towards the environment	Present	Non-present	Present
GHS Hazard Statement(s)	-highly toxic to fish and crustaceans with long-term side effects -Toxic if swallowed	-mild irritant (only)	-highly toxic to fish and crustaceans with long-term side effects -Toxic if swallowed

SOCIAL IMPACTS

- ✅ **Cost reduction**
- ✅ **No pollution** or **harmful effect** towards the environment
- ✅ Friendly towards **non-targeted organism**
- ✅ Introduction of **dual-pathway mechanism** to nanopesticide development
- ✅ Take advantage of **biomass** (coconut leaf) in the form of **waste**
- ✅ Achieve SDG **2nd** (Zero Hunger), **3rd** (Good Health and Well Being), **14th** (Life Below Water), and **15th** (Life On Land) Of United Nations(UN)

FURTHER RESEARCH

- Conduct **fumigant test** by using modified vial-in-vial method.
- Conduct **field test** on granular form and powdered form stored products.
- Conduct **cytotoxicity test** on non-target organisms.
- Test with **different ratio** of surfactant and essential oils.
- Conduct **stability study** of the nanoemulsion formulation.
- **SEM images analysis** on *T. castaneum.*
- **Extract AChE** of *T.castaneum*.

In conclusion, our research proves that the usage of nanoemulsions in conjunction with plant-based EOs enables a novel approach towards the control of *T. castaneum* while being cost-effective and eco-friendly.

N-ReoC is a novel, eco-friendly and cost-effective nanopesticide that benefits both mankind and the environment by tackling *T. castaneum* infestations, pollution issues and biomass waste issue.

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