(dedicated to u/ TemplarKnight the real IB MVP)

Antifungal Oils and their Modes of Action

Biology Extended Essay May 2020

Microbiology

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What is the effect of *Origanum compactum*, *Cymbopogon winterianus*, *Eucalyptus radiata*, *Mentha spicata* essential oils and hand soap on the rate of respiration and the growth of *Saccharomyces cerevisiae* (Baker's yeast) over a 48 hour period?

Contents

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1. Introduction

Antifungal agents can be fungicidal, that aim to kill the organisms, or fungistatic, that aim to stop their growth and reproduction (Pankey and Sabath, 2004). Certain essential oils act as growth inhibitors for microorganisms through similar processes to these drugs (fig. 1). Just like fungicidal drugs, the oils can dismantle the cell wall or attack the organelles of organisms (Nazzaro et al. 2017). The oils can inhibit the production of ATP, by either causing damage to the mitochondria or interfering with a metabolic pathway in cellular respiration, eg. Citric Acid Cycle. This lack of ATP will lead to slower growth or even cell death, for it is a necessary molecule in many non spontaneous metabolic processes inside cells. One theory explains that essential oils (EO) exhibit antimicrobial properties because plants synthesize the oils to protect themselves from pathogens (Swamy et al, 2016).

Essential Oil	**Effect on Cell**
Oregano	Action on fungal mitochondria
Lemon grass (Citronellal - Pubchem)	Cell wall, Inhibition of efflux pump (proton gradient across cell membrane necessary for nutrient uptake.)
Eucalyptus	Effect on cell growth and morphology
Spearmint (linalool and linalool - Pubchem)	Effect on membrane/wall, quorum sensing

Fig. 1 Table Summarizing mechanisms that Essential Oils use to impact the fungal cell *(Mechanisms from Nazzaro et al. 2017)*

The invention of antimicrobial drugs was a revolutionary step in science that has saved countless lives. Unfortunately, due to improper usage and frequent prescriptions, resistant organisms have developed. The bacteria, fungi or virus with genetic mutations that allow them to resist medicine can escape the host. Once this happens, the genes allowing for this resistance can be passed to other populations. This problem has become especially relevant in healthcare areas and hospitals.

One mechanism in microorganisms that display resistance is a pump that extracts the medicine from the target cell (see fig. 2). Drugs often aim to stop the functioning of an essential enzyme. Some cells change the drug's target enzyme's active site or they protect the targeted enzyme with another protein. Additional mechanisms include the synthesis of enzymes that directly impact the drug and can break the molecule down (Munita and Arias, 2016). The enzymes change the drug's structure so it can no longer reach its target.

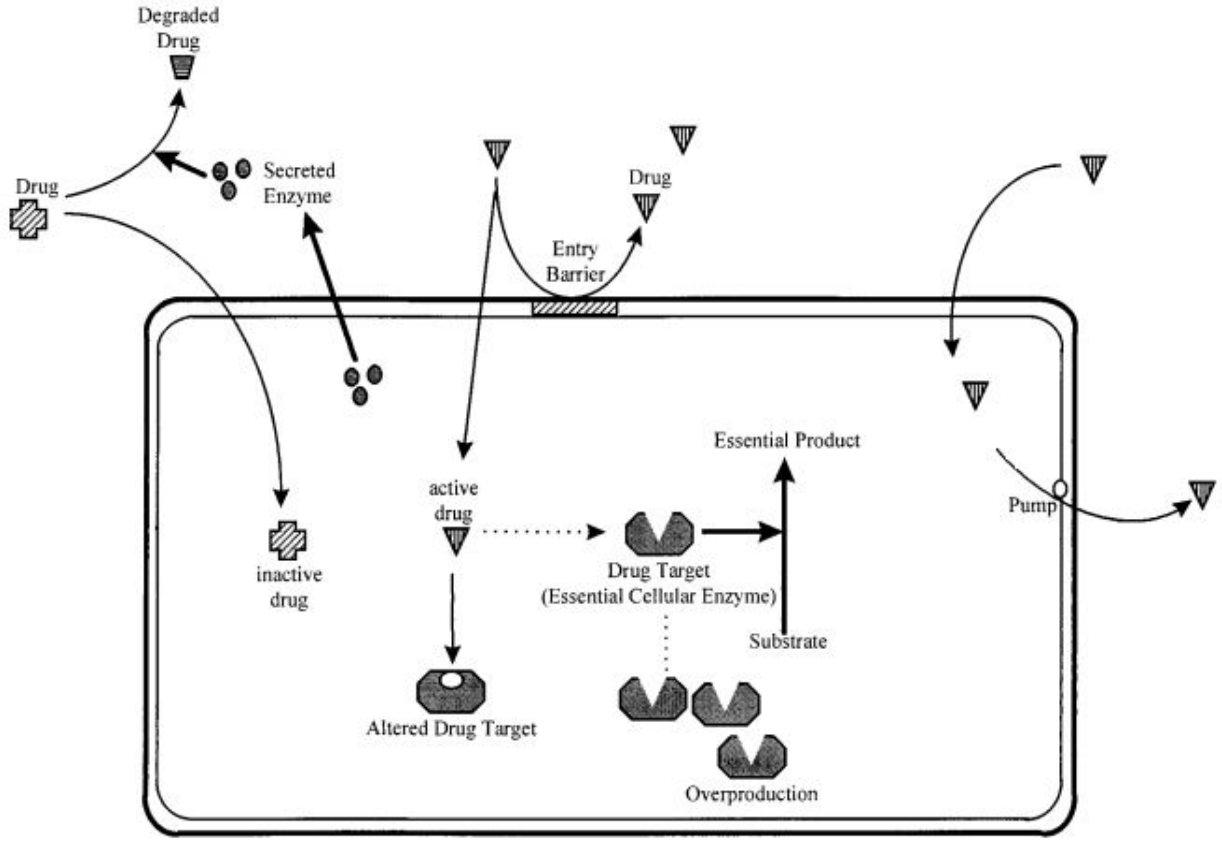

Figure 2. Diagram of mechanisms for antimicrobial resistance to drugs (Ghannoum & Rice, 1999)

This new resistance to drugs and antimicrobial chemicals can also be seen in agriculture. Some crop pathogens have become resistant to fungicides, because of constant use of the same pesticides with a single mode of action (i.e only targeting the cell wall). Reducing the usage of these chemicals and ensuring that they are partnered with another fungicide can prevent resistance in fungi. The two must not have similar mechanisms, so strains resistant against one fungicide will be killed by the other. This could lead to the rise in a dual-resistant organisms, but this is unlikely (Derek and Brent, 2007). Examples of chemicals that could be partnered with fungicides are essential oils.

They have been tested against species of Colletotrichum, a fungi that causes Anthracnose, a disease found in crops of South Korean pepper farms (Hong et al, 2015).

The focus of this investigation is to evaluate the efficiency of 4 essential oils (EO) and hand soap as antifungal agents. In experiment A, their ability to inhibit growth is tested through a disk diffusion test. The inhibitors create a zone of inhibition around the disk, which varies depending on their strength. In experiment B, their ability to inhibit respiration will be measured. This will be seen through the change in CO_2 production over time in the presence of different inhibitors. It would be expected that the soap results would not differ greatly from the control, because this hand soap is not antibacterial/fungal. Soap removes microorganisms from hands mechanically, through micelles and not chemically, like antibiotics or essential oils. It should therefore have no impact on the growth or respiration of fungi. It would be expected that EO targeting mitochondria will decrease the rate of respiration more than others. This is because ATP will only be synthesized through fermentation and not though respiration, which creates more carbon dioxide (CO_2) per glucose molecule.

1.2 Preliminary Tests

Tests were performed prior to data collection to determine the strength of the EOs and the soap. This would help indicate if the inhibition zones would be too large for the agar dish selected, allowing us to decide how many disks could be placed on one dish. For experiment B, dried yeast (used in baking) yielded more significant differences in CO_2 production than pre-ordered yeast suspensions. It was therefore deemed to be better suited for this experiment. The ordered suspensions were used in Experiment A, because they were presumed to be more sterile than the commercially available packets of dried *S. cerevisiae*. Both were the same species. The CO_2 probe gave an irregular curve, complicating the collection of data. A pressure probe was used instead.

2.1 Independent variable

Inhibitor placed with yeast in agar or yeast suspension: Oregano oil, Spearmint oil, Citronella oil (Java), Eucalyptus and Hand soap. Distilled Water was the control.

Commercially available oils were used. These were mixtures of different components and not single molecule solutions. *Origanum compactum* (Oregano), *Eucalyptus radiata* (Eucalyptus) and *Cymbopogon winterianus* (Spearmint) oils were bought from *Pranarôm. Mentha spicata* (Citronella) manufactured by *Puressentiel* was used.

2.2 Dependent Variable

Experiment A: Size of inhibition zones. These increase if the inhibitor is more effective because *S. cerevisiae* won't grow near it.

Experiment B : Rate of Respiration. Measured using a pressure probe (±0.1 kPa). Inhibitors that impact respiration will reduce CO_2 output (and therefore pressure) because it is a product of this process.

2.3 Controlled Variables for Experiment A

Incubation time of Yeast (48h) and incubation temperature (25 °C) was constant. Time fungi have to grow influences the size of the zones. Optimal growth temperature, 30°C, was not chosen (Salari, 2017). 25 °C was deemed safer, because potential human pathogens could be grown at temperatures resembling those of the human body. (Nuffield Foundation)

Volume of *S. cerevisiae* suspension used (200µL ±0.1) and concentration of yeast suspension (10g/L). Inhibitors would be less effective if a larger number of yeast was present. Suspension was shaken up before each trial to homogenise. Same

species and strain of *yeast* (ordered from Sordalab) was used for every trial. Non-identical species would interact differently with the inhibitors.

Keeping the same agar recipe (see appendix) will keep the pH, the presence of certain nutrients and other factors influencing growth constant. The same *"Nutrient agar"* was ordered each time from technician.

Size of paper diffusion disks (7mm ±1) was kept the same, because a larger disk could lead to a larger inhibition area.

The same Volume (40 µL ±0.1) and Concentration of inhibitors was used. Oils and soap were used directly from containers without the addition of water (assumed to be pure). A diluted inhibitor would be less effective and would create an unequal comparison.

2.4 Controlled Variables for Experiment B

Time the organisms were exposed to the oils and soap was constant (5 minutes). The solutions were left for equal periods after the inhibitor and glucose were added. Inhibitors acted on the yeast for the same length of time.

Water bath temperature (30 °C ±1) A higher temperature could lead to more metabolic activity or a decrease if denaturation of enzymes occurred. This would make yeast produce more CO_2, because they would respire more. Suspension was kept at 30°C, because it is the optimum growth temperature of S. cerevisiae (Salari, 2017). It did not matter if it was near human body temperature, because no pathogens would have time to grow.

Concentration of inhibitor ("pure"), yeast suspension (50g/L ±0.1) and glucose solution(20 g/L ±0.1) were the same. Volume of inhibitor (5 mL ±0.1), yeast suspension (15 mL ±0.1) and glucose solution (5 mL ±0.1) were constant. Having more yeast would increase the CO_2 produced because more organisms would be respiring. If the concentration of inhibitor increased, the CO_2 output could decrease because more phytochemicals (the EO) could damage the cells. Glucose is used as an energy source instead of a dimer or polymer, to reduce the time taken to metabolise.

3.1 Procedure for Experiment A

The Kirby Bauer disk diffusion test is an official method for testing antimicrobial activity in many clinical laboratories (Balouiri et al, 2016). *S. cerevisiae* was chosen because it is easily available and non pathogenic. It is also an organism widely used for studying eukaryotes, making it easier to compare results. (Shen et al, 2014)

Prior to the experiment, a yeast solution (10g/L) was ordered. Nutrient Agar was prepared by technician. Sterile environment was prepared with a Bunsen burner and disinfectant wipes.

Agar was inoculated with 200 µL ±0.1 yeast solution and evenly spread. This was allowed to dry until the surface was not reflective. Metal pincers were passed through Bunsen flame after every interaction to sterilise them. 40 µL±0.1 of distilled water was placed on filter-paper disk. Filter-paper disk was then allowed to dry for a few seconds before being placed on the agar (fig 3). Two more disks were added with the same process. Only 3 disks were placed on each agar dish to avoid inhibition zone overlap. The same process was repeated for the EO and soap.

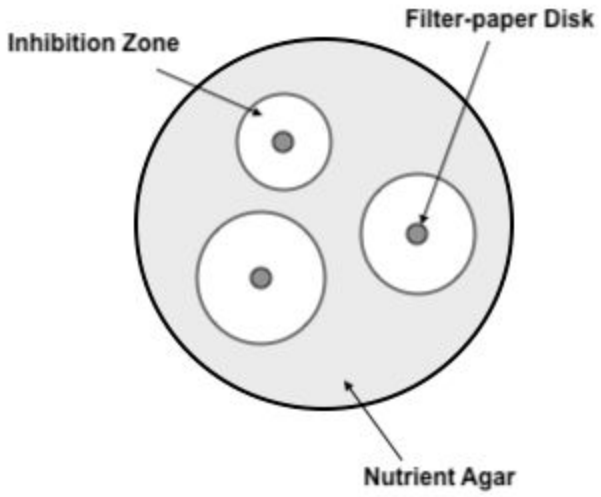

Figure 3 - Diagram of Experiment A - Authors Image

The petri dishes containing the yeast were set aside upside down to avoid problems with condensation. They were placed in an incubator at 25°C for a period of 48h. The zones of inhibition were then measured over a lit-up surface to facilitate identification of their radius. The zones were often not clear or were larger than the expected boundaries, so an estimation was made. If only one side of the circle was clear, the radius was multiplied by 2. Smallest radius was always taken. If any disks had moved the zones were estimated as shown in figure 4.

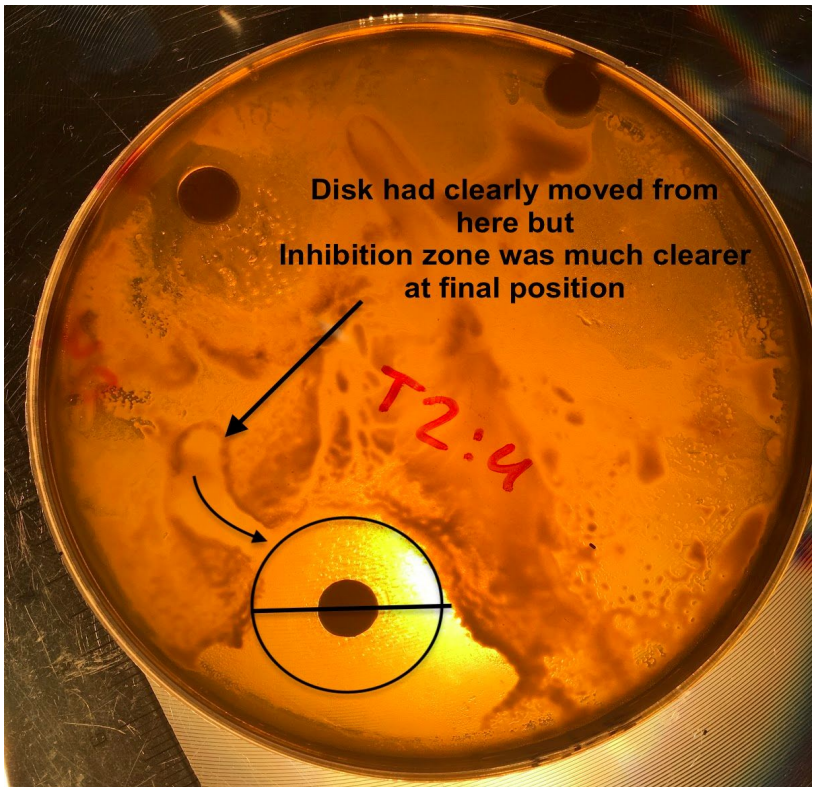

Fig 4. Eucalyptus trial where disk moved and zone was estimated

Note : methylene blue dye was placed on yeast cultures to better identify inhibition zones by staining the walls of the fungi. This did not work because the dye stains dead cells. (Sharga et al, 2017)

3.2 Procedure for Experiment B: Inhibition of respiration

A yeast solution (50g/L ± 0.1) was made up from pure, dried baker's yeast and bottled water. Test tubes were set up with 5 mL ± 0.1 of an inhibitor (or additional water for control), 15 mL ± 0.1 of yeast solution and 5 mL ± 0.1 glucose solution (20 g/L ± 0.1). This was shaken 3 times and left to sit for 1 minute before being transferred to a hot bath at 30 C ± 1 for 4 minutes. This was done to allow the inhibitor to take action and to allow the dried yeast to start respiring. Glucose was used, because it was metabolised faster than a dimer or polymer.

After being removed from the water-bath, a pressure probe was linked up to the test tube (fig 5). The joints of the tubing were sealed to ensure minimal leakages. Pressure was measured over 40 seconds. This was repeated for every inhibitor.

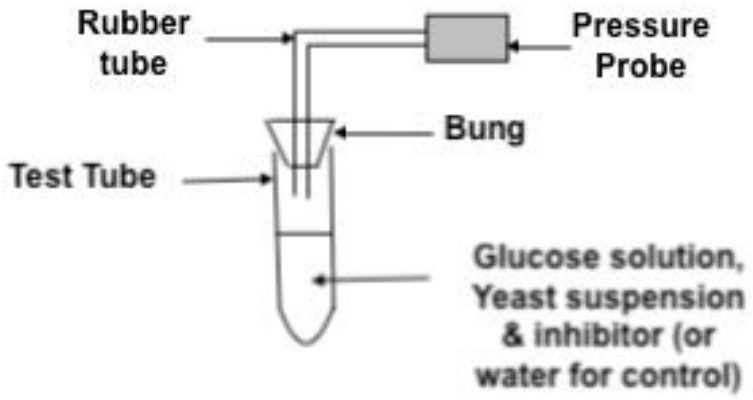

Figure 5. Experiment B diagram - Author's Image

Note: The author averaged a reaction speed of 238ms using an online program (Human benchmark). The uncertainty associated with time in experiment B was minimal.

3.3 Ethical and Safety Issues

Contaminated agar was safely disposed of by technician after trials. Unknown microbes or potential pathogens could have been grown. The temperature of the incubator was kept under the human body temperature. No antifungal drugs were used because resistant organisms could be cultured. The highly flammable material was kept clear of the flame to avoid combustion.

4.1 Statistical Tests and Analysis

For experiment B, it was observed that the standard deviation of the control results greatly decreased (from 1.79 to 0.690) when the result from test 5 (see appendix) was omitted. It was left out when calculating the error bars in the graphs to reduce overlap with the inhibitors. However, this result was not taken out during the ANOVA statistical test because 5 values are needed. This selecting of data was performed to facilitate comparisons between variables. It was assumed that the last test of the control was an error, because the control had the largest standard deviation. This reflects a degree of uncertainty, because the data had to be adjusted for the tests to be run. This must be taken into consideration when evaluating the data.

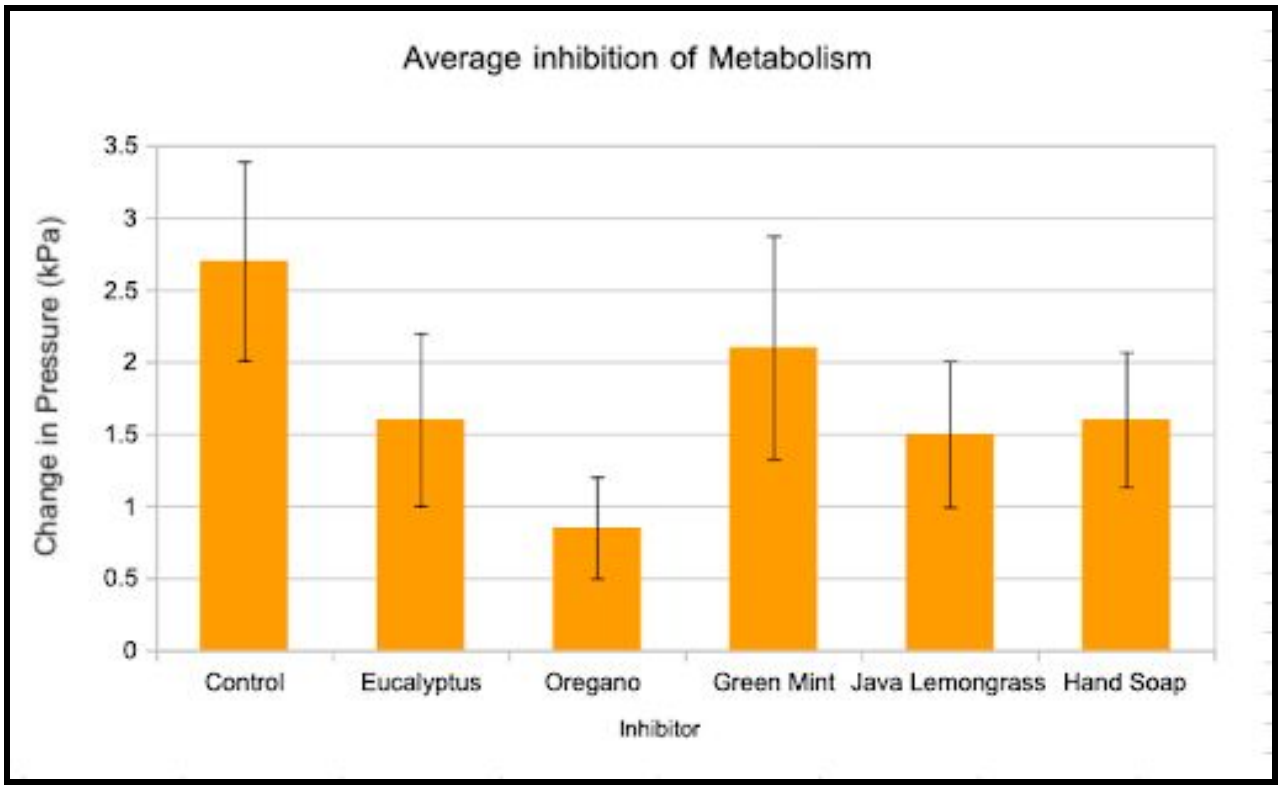

Fig 6. Experiment B - Rate of respiration in the presence of inhibitors - Error Bars represent standard deviation

The biggest change in pressure shows the production of the most CO_2. Non overlapping standard deviation error bars are a sign of potential significant difference between results. In figure 6, the highest average was the control, which was expected, seeing as non-inhibited respiration would lead to the highest levels of CO_2 produced. The oregano had the lowest respiration rates. The average for every inhibitor, including soap, was lower than the control. However, the only non-overlapping results are oregano's relative to the control, green mint and soap. This shows how the only inhibitor that displayed inhibition of respiration was oregano because it was significantly different from the control. It was also significantly more efficient at blocking respiration than green mint and soap, who had the second and third highest averages respectively.

Many of the error bars are only overlapping slightly, so an additional statistical test was used.

An ANOVA with a post hoc Bonferroni and Holm statistical test was performed on the metabolism results using an Excel calculator. The ANOVA test allows us to verify the statistical difference between at least 2 groups. The Bonferroni and Holm tests will allow us to check for significant differences between pairs of data relative to the control (McDonald et al, 2014). This would indicate which inhibitors were different from the control and therefore could be considered to have influenced respiration.

A F-value of 1.961 with a corresponding p-value of 0.121 were found. No significant difference was found for a level of significance of $p < 0.05$. Despite this, a post hoc test was performed to check for differences between groups and the control. The ANOVA and post hoc tests do not always match and differences between groups may be missed if data is dismissed after an insignificant ANOVA test. (Chen et al, 2018)

Inhibitor vs Control	T-statistic	Bonferroni p-value	Bonferroni inferfence	Holm p-value	Holm inferfence
Eucalyptus	1.7778	0.44053	insignificant	0.26431	insignificant
Oregano	2.9450	0.03534	insignificant	0.03534	$p < 0.05$
Green Mint	0.9605	1.7319	insignificant	0.34639	insignificant
Lemongrass	1.9464	0.31702	insignificant	0.25362	insignificant
Soap	1.5107	0.71962	insignificant	0.28785	insignificant

Fig 7. Table for Bonferroni and Holm test results - Screenshot from Excel - Calculations in appendix

The Holm test (fig 7) shows that the results for oregano are significantly different to the control. Therefore, oregano can potentially impact CO_2 production and consequently the respiration rate of *S. cerevisiae*. This supports the literature that states that only oregano acts on the mitochondria of the cell. The Bonferroni test shows no significance. The contradictions between tests reduces the reliability of these results and the conclusions drawn from them, even if the error bars and the Holm test are in agreement.

After establishing that oregano is the only inhibitor that impacts respiration, the efficiency of the inhibitors against *S. cerevisiae* was compared. Is the inhibition of respiration the most efficient mechanism to reduce growth in this species?

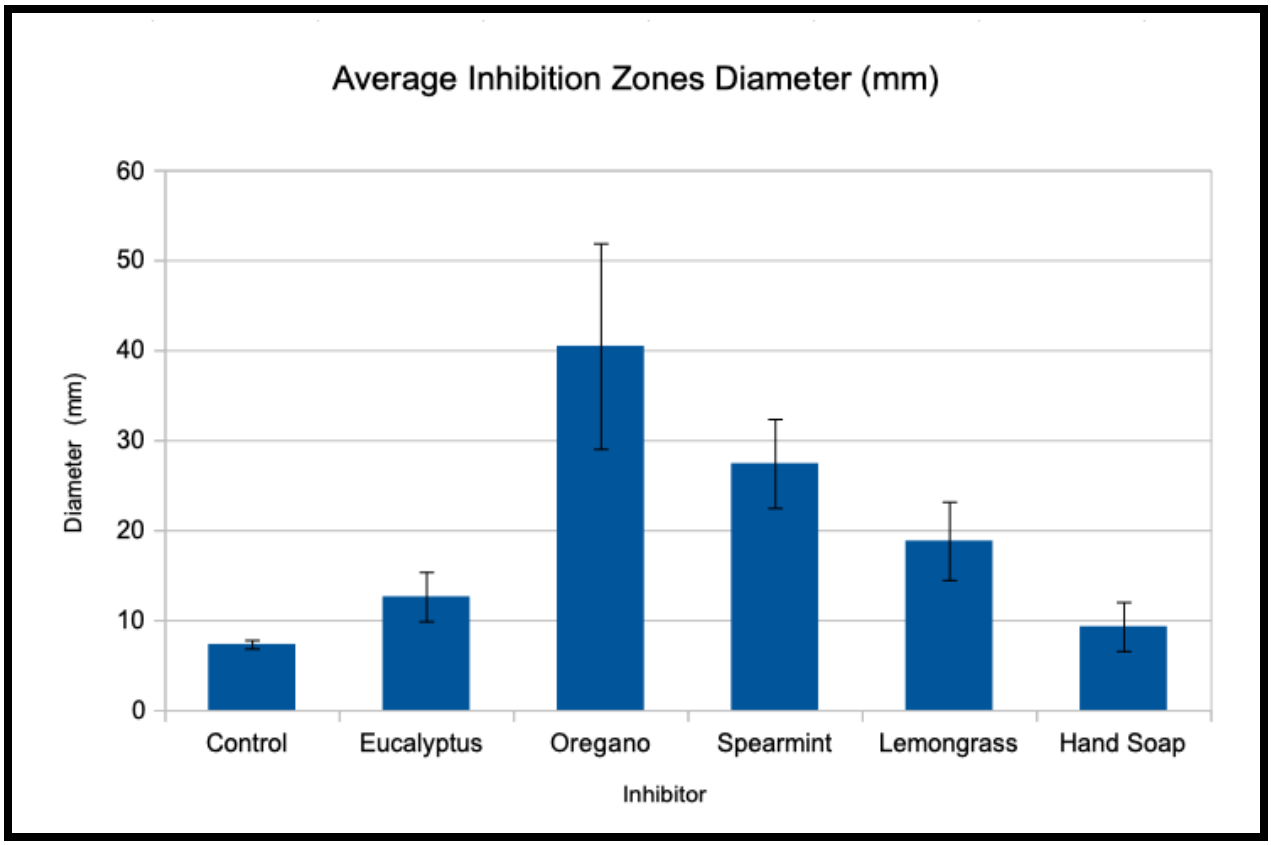

Figure. 8 Experiment A : Results for Disk diffusion test - Error Bars represent Standard Deviation

The control has the lowest inhibition zone. Figure 8 shows that the soap, the inhibitor with the lowest average of 9.3, has error bars overlapping with the control. This shows how it does not display antifungal properties. There is no overlap between any of the EO and the control, showing that they all have an impact on the growth of *S. cerevisiae*. Oregano possesses the greatest antifungal capacities (at 40.5mm ± 1) if the standard deviation of the results is not considered. However, the error bars overlap between oregano and spearmint, showing that there is no significant difference between the two EO. Oregano is a better inhibitor than eucalyptus (12.6 mm ± 1) and

lemongrass (18.8mm ± 1). An additional statistical test was performed to verify significance.

Inhibitor vs Control	Bonferroni and Holm T-statistic	Bonferroni p-value	Bonferroni Inference	Holm p-value	Holm Inference
Eucalyptus	1.9397	0.2864061	insignificant	0.1145624	insignificant
Oregano	13.2757	0	p < 0.05	0	p < 0.05
Green Mint	8.2307	1.26E-010	p < 0.05	1.01E-010	p < 0.05
Lemongrass	4.6034	0.0001157	p < 0.05	6.94E-005	p < 0.05
Soap	0.7685	2.2265748	insignificant	0.445315	insignificant

Fig. 9. Bonferroni and Holm test results showing significant different to control

Another ANOVA with a post hoc Bonferroni and Holm test was performed as before. A F-value of 49.14 and a corresponding p-value lower than 0.05 resulted. This indicates the presence of a statistically significant difference. The Bonferroni and Holm test (fig 9) show the significant difference relative to the control. Only Eucalyptus and soap show no significant difference showing that they do not inhibit growth. There is no disagreement between any of the tests except for the eucalyptus. Therefore, oregano, green mint and lemongrass show stronger antifungal properties. Eucalyptus shows more moderate inhibition.

R- Value correlation

A R-value correlation was performed to ensure there was no inverse correlation between the CO_2 measured and the inhibition zone size. This would help confirm that

the oregano was blocking respiration and not just killing the yeast proportionally faster than the others. A high correlation signifies that low CO_2 production was due to less organisms being present and not the inhibition of respiration.

The R-value calculated was -0.67, showing that there is a weak negative correlation. This indicates the possibility that the inhibitors are not blocking respiration and are simply killing the organisms. If this is the case, then the CO_2 output goes down because there are fewer yeast present and not that the mitochondrial function is disrupted by the oregano. This would render previous statistical tests on experiment B irrelevant. It is unlikely because, as seen previously, the results support the literature.

5.1 Evaluation

sheesh data analysis is boring sometimes... ANOVA tests are rlly easy to do online and they look good on your paper, but ask your supervisor...

A major issue was the aseptic technique. The lab where the experiments were performed did not have the standard level of sterility (no laminar airflow). Therefore, ensuring that no contaminants had entered the growth medium was impossible. These foreign species could be more or less resistant to the inhibitors and could even have a competitive relationship with *S. cerevisiae*. Even if repeated trials showed similar results, it is highly possible that they were contaminated with the same microorganisms because dishes were exposed to similar environments. However, after measurements were taken, a sample was taken from petri dishes where growth seemed irregular or contrasting to other colonies (fig.10) and examined under the microscope.

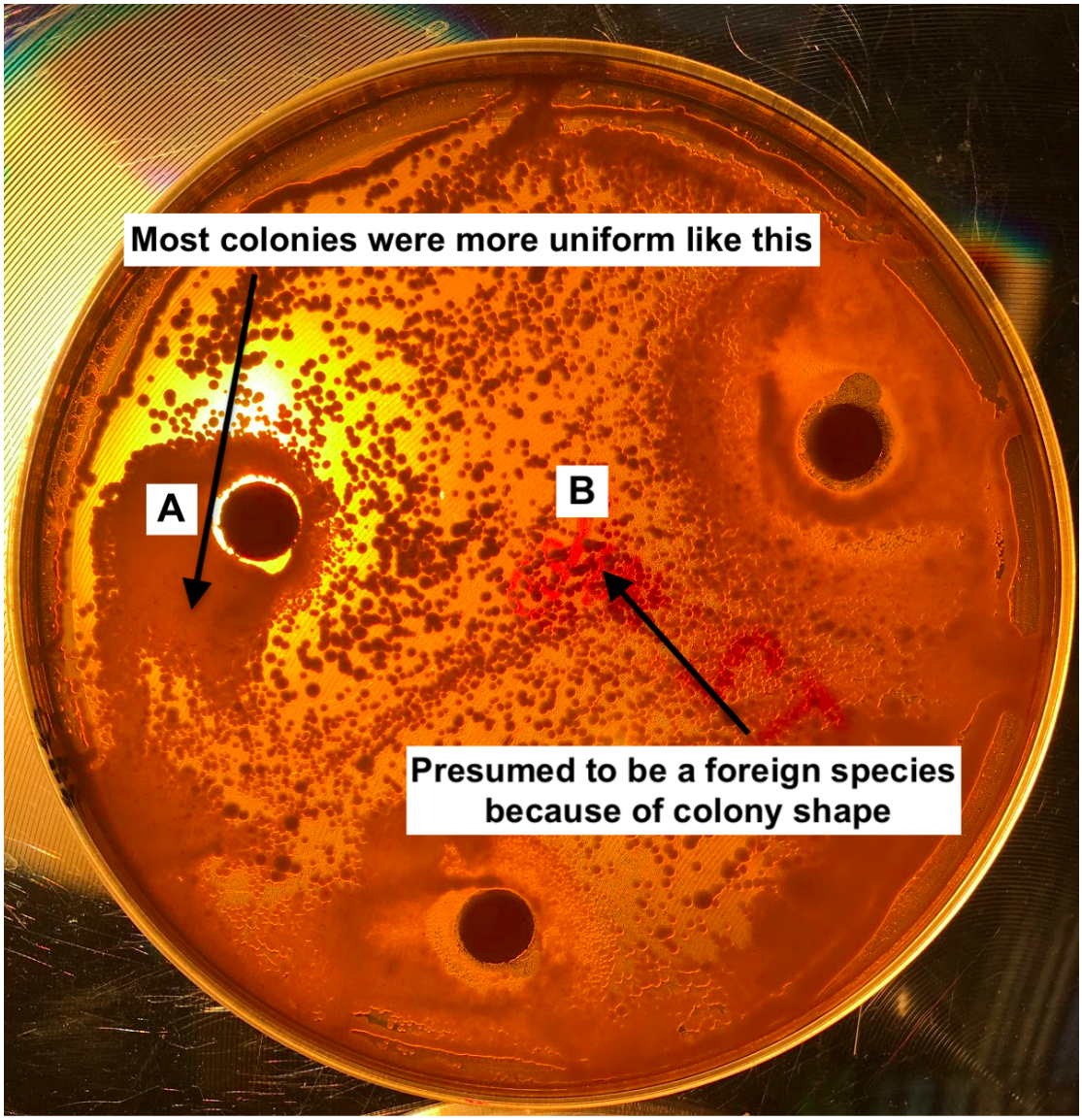

Fig 10 - photographed results from soap trial where colonies were non-homogenous

No contrast was found between microorganisms in colonies A and B shown above. This test would not identify all species but it did add some degree of certainty to the sterility of the tests. It was assumed that yeast colonies didn't grow enough to connect, due to uneven spread of the yeast solution. The colonies could have been left to grow before

the disks were applied. This could allow for more clear inhibition zones and a more even growth of yeast.

Some zones were too big and the dilution of the essential oils to a lower concentration could have been beneficial to obtain smaller and more clear cut inhibition areas. It was also deemed more efficient to have only 3 disks per agar dish, so that inhibition zones would not overlap but less agar would be wasted (compared to having 1 disk per dish).

The McFarland standard is used to ensure that the same “concentration” of yeast suspension is used. It was not used, but yeast were ordered at a specific concentration. It was deemed more efficient and sterile than creating a solution from isolated colonies. This must be considered when comparing data with literature because a different number of *S. cerevisiae* could be present when agar was inoculated. Additionally, the oils were assumed to be pure (no labeling on containers was found). This could lead to differences between collected and literature data.

The eucalyptus oil was the most volatile oil. It evaporated the fastest when a drop from each EO was placed on a dish. The quantity that stayed on the disks could impact the size of the inhibition zones. To minimise this, the “agar dilution test”, where inhibitors are dissolved into the agar, could be used. A drop of the yeast is placed on the agar instead of being spread. The disk diffusion test was used because the agar dilution test is more time consuming and allowed for more chances of contamination (Gaudreau,

2007). The agar dilution test is used to determine the minimum concentration of the inhibitor needed to stop growth. The inhibitor with the lowest minimum inhibitory concentration (MIC) would be the most efficient antifungal agent.

5.2 Source Evaluation and Comparison

The Disk Diffusion procedure allowed for easier comparison of data, because it is commonly used. No data on respiration inhibition of these oils could be found, so values were compared to the literature modes of action instead.

It is challenging to precisely compare collected data with other sources due to the experimental conditions varying from test to test. This has also been reported in other studies, where different incubation times, EO concentrations and yeast strains are used. This leads to a difficulty in data comparison (Erguden & Konuk, 2019). The concentration of molecules responsible for growth inhibition (i.e Eugenol) can also vary in plants grown in different conditions (Lingan, 2018). The inhibition zones were unclear or non-circular for some trials, making the determination of a precise radius difficult.

Any literature data discussing antifungal EOs was initially used to compare because the inhibitors were assumed to act similarly on eukaryotes with related ultrastructures. Data was selected from *S. cerevisiae*, because the difference in resistance between organisms for the same EO proved to be greater than anticipated.

Some sources were assumed to be more reliable because they were found in databases like the National Center of Biotechnology Information (NCBI) where works are reviewed. Articles that used an appropriate structure (Introduction, Method, Results, Discussion), cited their sources, presented information about the authors and the institutions were considered reasonably reliable. However, certain studies could be influenced by conflicts of interest with financial investors (Romain, 2015). The most recent sources were chosen when possible, because they were assumed to contain the newest information. The only article with unidentified authors was from “DrugBank”. However, this resource has been evaluated by other researchers (Wishart et al, 2007)

5.2 Discussion and Conclusion

Having taken into account the possible errors with the procedure, the literature values differ from those collected (fig. 11).

Essential Oil	**Inhibition Zone diameter (mm)**		**Authors / Sources**
	Experimental Value	***Literature Value***	
Eucalyptus	12.6	15.0	Tyagi et al, 2014
Oregano	40.5	30.0	Çoşkun et al, 2016
Spearmint	27.4	16.0	Çoşkun et al, 2016
Lemon grass	18.8	28.0	Helal et al, 2006

Figure 11 Table Summarising literature values against experimental values for EO

This is expected, because of the number of errors that occurred. The highest and lowest values, oregano and eucalyptus respectively, are the same in the literature and

experimental values. The standard deviation indicated that the EOs possess antifungal properties. However, the ANOVA with post hoc Bonferroni and Holm test indicated that eucalyptus was not antifungal. It is the EO with the smallest inhibition zone (both in the literature and experimentally). This can explain the result of the statistical test. Determining the most effective oil is difficult due to some overlap between data sets. However, it could be suggested that oregano and spearmint exhibited the largest antifungal properties, because they have the largest means. This does not support the literature data, because spearmint had the second lowest inhibition zone diameter of 16mm. We can note that spearmint, unlike oregano, had overlapping error bars with lemongrass.

Soap has a higher average than the control. 1 of the 23 constituents of the hand soap, Methylchloroisothiazolinone, was found to inhibit fungal growth (Drugbank). The ANOVA statistical test and the error bars show that there was no antifungal activity in the soap. We can conclude that only the EO showed antimicrobial properties.

For experiment B, oregano had the lowest amount of CO_2 produced. It is the only inhibitor that shows some significant differences to the control (seen in post hoc test and standard deviation). Oregano is the only EO to inhibit growth by damaging the mitochondria. However, the yeast were assumed to not have been killed, because the time that the inhibitors were in contact with the organisms was minimal. The respiration inhibition should not be proportional to the growth inhibition if this is the case. The

R-value of correlation (-0.67) suggests this could be possible, meaning experiment B does not demonstrate the inhibition of respiration.

Regular hand soap washes away microorganisms by creating micelles. The error bars overlap, showing that this difference could be insignificant. There is much uncertainty in the results collected because of the contradictory statistical tests, as well as the potential correlation between growth and respiration results.

An additional measurement could be the change in extracellular pH after the addition of EO. It would indicate damage to the cell membrane, because of the differences in pH inside and outside the yeast (Erguden & Konuk, 2019). Changes in the duration of the cell cycle stages could also indicate the action of EO on cell growth or replication (Kono et al, 2016). Inhibition of biofilm formation is also interesting. Qualitative analysis using red congo agar, which changes colour in the presence of biofilms, could aid with this analysis (Kırmusaoğlu 2019). These measurements would provide additional evidence to the EO's modes of action proposed by the literature.

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Appendix

Agar Recipe : 0.5% Peptone, 0.3% beef extract/yeast extract, 1.5% agar, Distilled water, pH is adjusted to neutral (7.4) at 25 °C.

28 g of nutrient agar powder suspended in 1 litre of distilled water (Aryal, 2018)

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Raw Data

Experiment A : Inhibition zones

Table showing results for each trial (averages and standard deviation also shown)

	Inhibition Zone Diameter (± 1mm)													
Inhibitor	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9	Trial 10	Trial 11	Trial 12	Average	σ Standard deviation

Control	7	7	8	7	7	7	8	7	7	8	8	7	7.33	0.471
Eucalyptus	12	10	x	10	**x**	**x**	17	11	**x**	16	15	10	12.625	2.736
Oregano	35	30	25	40	40	20	47	51	55	46	56	**x**	40.455	11.42
Spearmint	27	26	34	30	36	28	25	32	24	23	17	27	27.416	4.941
Lemongrass	20	16	**x**	24	22	15	18	19	14	22	11	26	18.818	4.345
Hand Soap	17	x	**x**	8	8	10	8	7	8	9	8	10	9.3	2.722

Diameter of disk : 7 mm
X → unclear inhibition zone

Experiment B : Respiration Inhibition

Table showing results for each trial (averages and standard deviation also shown)

	Difference in pressure (kPa) after 5 mins for 40 secs						
Essential Oil / Inhibitor	**TEST 1**	**TEST 2**	**TEST 3**	**TEST 4**	**TEST 5**	**Average**	**Standard Deviation**
Control	2.16	0.93	1.57	2.79	6.05	2.7	1.79
Eucalyptus	2.33	1.98	1.33	0.58	1.69	1.582	0.600
Oregano	0.29	1.16	0.69	1.28	0.82	0.848	0.352

Spearmint	3.43	2.5	1.46	1.4	1.69	2.096	0.774
Lemongrass	1.62	2.33	0.93	1.51	0.99	1.476	0.507
Hand Soap	1.97	1.22	2.55	1.45	1.56	1.75	0.468

ANOVA 1 way statistical test for experiment B

	sum of squares	degrees of freedom	mean square	F Value	p-value
treatment	9.6937	5	1.9387	1.9610	0.1213
error	23.7276	24	0.9886		
total	33.4213	29			

Treatment	Control	Eucalyptus	Oregano	Green Mint	Lemongrass	Hand Soap	Total
number of trials	5	5	5	5	5	5	30
sum	13.5000	7.9100	4.2400	10.4800	7.3800	8.7500	52.2600
mean	2.7000	1.5820	0.8480	2.0960	1.4760	1.7500	1.7420
sum of squares	52.3820	14.3107	4.2166	24.9626	12.1784	16.4079	124.4582
sample standard deviation	1.9957	0.6703	0.3940	0.8655	0.5669	0.5233	1.0735
standard dev of means	0.8925	0.2998	0.1762	0.3871	0.2535	0.2340	0.1960

Screenshot of formulas used in ANOVA test

One-Way ANOVA Table					
Source	**Degrees of Freedom** **DF**	**Sum of Squares** **SS**	**Mean Square** **MS**	**F-Stat**	**P-Value**
Between Groups	$k - 1$	SS_B	$MS_B = SS_B / (k - 1)$	$F = MS_B / MS_W$	Right tail of $F(k-1,N-k)$
Within Groups	$N - k$	SS_W	$MS_W = SS_W / (N - k)$		
Total:	$N - 1$	$SS_T = SS_B + SS_W$			

ANOVA for experiment A

Treatment →	A	B	C	D	E	F	Pooled Total
observations N	12	8	11	12	11	10	64
sum ∑xi	88	101	445	329	207	93	1263
mean $\bar{x}$	7.3333	12.625	40.4545	27.4167	18.8182	9.3	19.7344
sum of squares ∑x2i	648	1335	19437	9313	4103	939	35775
sample variance s2	0.2424	8.5536	143.4727	26.6288	20.7636	8.2333	172.2299
sample std. dev. s	0.4924	2.9246	11.978	5.1603	4.5567	2.8694	13.1236
std. dev. of mean SE$\bar{x}$	0.1421	1.034	3.6115	1.4897	1.3739	0.9074	1.6405

source	sum of squares SS	degrees of freedom ν	mean square MS	F statistic	p-value
treatment	8778.5624	5	1755.7125	49.1482	1.11E-016
error	2071.922	58	35.7228		
total	10850.4844	63			

Between Groups Degrees of Freedom: $\mathbf{DF = k - 1}$, where $\mathbf{k}$ is the number of groups

Within Groups Degrees of Freedom: $\mathbf{DF = N - k}$, where $\mathbf{N}$ is the total number of subjects

Total Degrees of Freedom: $\mathbf{DF = N - 1}$

Sum of Squares Between Groups: $\mathbf{SS_B} = \sum_{i=1}^{k} \mathbf{n_i} (\bar{x}_i - \bar{x})^2$, where $\mathbf{n_i}$ is the number of subjects in the i-th group

Sum of Squares Within Groups: $\mathbf{SS_W} = \sum_{i=1}^{k} \mathbf{(n_i - 1)\, S_i^2}$, where $\mathbf{S_i}$ is the standard deviation of the i-th group

Total Sum of Squares: $\mathbf{SS_T = SS_B + SS_W}$

Mean Square Between Groups: $\mathbf{MS_B = SS_B / (k - 1)}$

Mean Square Within Groups: $\mathbf{MS_W = SS_W / (N - k)}$

F-Statistic (or F-ratio): $\mathbf{F = MS_B / MS_W}$