Testing antimicrobial effects of tea tree oil using *S. cerevisiae* and bread mold

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Abstract Tea tree oil (TTO) is a naturally-occurring chemical extracted from the leaves of the narrow-leaved paperbark tree. It is well known as an antiseptic, but its potential as a natural household cleaning agent has not been thoroughly examined. We were interested in examining the anti-fungal properties of TTO, especially on common bread mold, spores of which are common to households. This study used fresh-baked white bread as media to observe the effects of TTO at 0.06% and 0.24% concentrations on mold spore formation and 0.25% TTO on yeast fermentation. It was concluded that TTO can inhibit mold growth at 0.06% concentration but did not reduce ($p=0.07$) yeast CO_2 production even at 0.25% concentration. The data highlights the efficacy of TTO against household mold and, based on our yeast results, is potentially non-toxic at low concentrations. More studies are required with a variety of mold species and concentrations of TTO to better understand its use as a cleaning agent.

INTRODUCTION

ATea Tree Oil (TTO) is extracted from the tree Melaleuca alternifolia, found in Australia (Hammer et al., 2006). When used topically, TTO has exhibited antimicrobial and antifungal effects and is an antiseptic to treat wounds (Low et al., 2017). The presence of household mold exposure has previously been linked to various respiratory issues, such as asthma in children (Zhang et al., 2021). Thus, there is a need for antifungals that are safe to use in household settings, and TTO is a natural alternative to harsher chemical agents that are currently being used. Moreover, the use of tea tree oil as a film coating over computer keyboards has been shown to significantly decrease bacterial loads in hospital settings (Melegari et al., 2021). This indicates potential unexplored applications of TTO, especially as an eco-friendly alternative to chemical cleaners.

Most common household microbes are mold that grows under high humidity and temperature (Vagelas et al., 2011) and commonly belong to the genera Aspergillus, Mucor, Fusarium, and Rhizopus (Garcia & Copetti, 2019). TTO has previously been tested as an effective antifungal that can completely inhibit A. fumigatus indoors, in both liquid and vapour forms via direct contact (Rogawansamy et al., 2015). However, its properties as a potential household cleaning oil have not been thoroughly evaluated.

TTO is made up of multiple phytochemicals. The most active component is terpinen-4-ol, which causes the membrane of the target organisms to become more permeable (Rogawansamy et al., 2015). This causes irreversible damage to the bacterial cell membranes, resulting in the loss of essential cell constituents and eventually death (Shi et al., 2018). The minimum inhibitory concentration of TTO against a wide range of fungi was found to be 0.004% to 0.25% (Hammer et al., 2002).

Due to COVID-19 restrictions on lab activities, we devised a method to test out the efficacy of TTO on bread mold and yeast under controlled conditions in situ. White bread was used as a growth media for growing mold and testing the inhibitory properties of varying concentrations of TTO in four separate experiments. The efficacies of the TTO concentrations were further evaluated on gas production by *Saccharomyces cerevisiae*, as a measure of toxicity.

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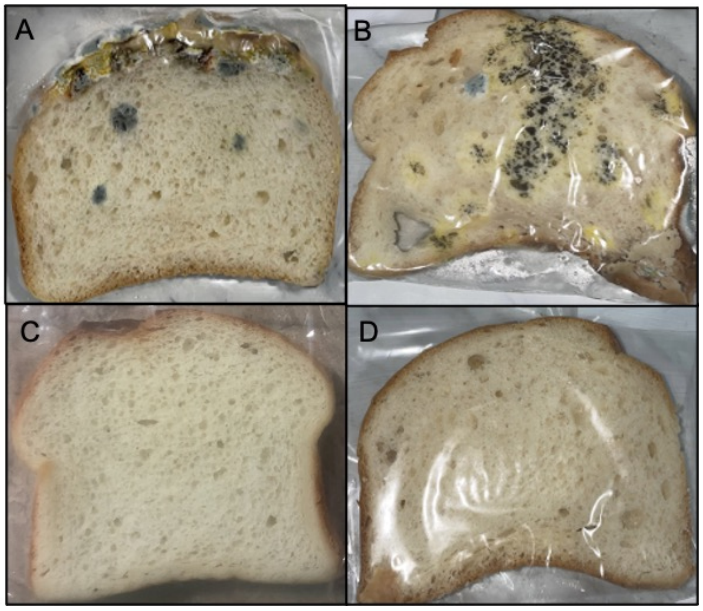

Figure 1 White bread exposed to different treatments after 7 days when placed in dark, humid environment at ~25°C. A = no treatment; B = water; C = 0.06% TTO; D = 0.24% TTO.

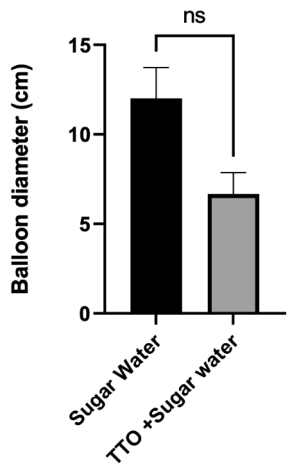

Figure 2 Bars represent the average balloon diameter ± standard error mean (SEM). Amount of gas produced by *Saccharomyces cerevisiae* (yeast), captured in the balloon, was unaffected by 0.25% TTO (p=0.07) as determined by unpaired Student's t-test. The data is the average of three independent replicates. The diameter of the balloon for all associated negative controls (water + sugar, water + yeast, water + sugar + 0.25% TTO, water + yeast + 0.25% TTO) was 0 cm.

Table 1 Average number of CFU on white bread when exposed to 0.06% and 0.24% TTO after 7 days under dark, humid conditions at ~25°C. Colony Forming Units (CFU) are reported as an average ± standard error mean (SEM) of 4 replicates. TTO = Tea Tree Oil; NG = no growth.

Treatment	Average number of CFU ± SEM
Bread alone	12 ± 0.6
Bread + water	17 ± 0.8
0.06% TTO	NG
0.24% TTO	NG

METHODS

TTO Effect on Mold Growth

The effect of TTO on the growth and spread of mold spores was analyzed. In-store (Fortinos) baked white bread was used as growth media and placed under conditions that will promote the growth of common bread mold, further explained below.

The number of Colony Forming Units (CFU) was compared in the presence of TTO.

TTO (Holista brand, 50mL bottle) media was diluted to 0.06% and 0.24% (v/v) and dissolved using water to obtain a total volume of 10 mL. Ziplock bags were labeled, and the bread was cut into approximately 8 cm × 5 cm × 1.25 cm slices and placed in each bag. A 100 mL refillable plastic spray bottle was used to spray 10 mL of each TTO concentration onto separate bread slices inside the Ziplock bags, which were then sealed. Two negative controls were prepared by spraying 10mL of water onto one slice of bread, while another slice was placed in a bag without any liquids. The positive control was the bread sprayed with 0.24% TTO, as this was previously shown to be the highest concentration that inhibits mold (Hammer et al., 2002). All samples were sealed in Ziplock bags and placed in a dark room at ~25°C which was maintained by monitoring house thermostats. The growth of mold was observed every day for 7 days.

TTO Effect on Yeast Growth

The effect of 0.25% TTO as a toxicant was tested on *S. cerevisiae*. A solution of yeast (Fleischmann's quick-rise instant yeast) + sucrose (table sugar) was prepared to allow fermentation to occur, and the amount of gas produced was measured. In the presence of 0.25% TTO, the gas production was compared with yeast + sugar solution without TTO to see the effects of TTO on yeasts' ability to produce measurable gas. More details are described below.

The yeast solutions were prepared using 100mL of water and 2% (w/v) of yeast and 2% (w/v) of sugar. The test samples were placed in bottles and were prepared by adding 5 drops of TTO to make 0.25% TTO. This method of dilution was found to be simpler, as 20 drops were measured to equal 1mL, therefore 5 drops were used to make 0.25% (v/v) TTO in 100mL of water. The controls were set up alongside the test samples with the same 100mL volume. Negative controls were bottles with water + sugar, water + yeast, water + sugar + TTO, and water + yeast + TTO. The positive control was water + sugar + yeast. All samples were then mixed, and the bottle opening was covered with a balloon to collect any gas produced from the mixture. The bottles were placed in a hot water bath for 40 minutes

to maintain an average temperature of 45°C. The diameters of the balloon after 40 minutes were recorded to analyze the amount of gas produced in the fermentation process.

Statistical Analysis

The effect of TTO concentration on gas production by *S. cerevisiae*, grown in sugar water, was analyzed using an unpaired Student's t-test with GraphPad Prism 9.0.

RESULTS

Mold CFU on bread shown in Figure 1 were counted following 7 days of exposure to various treatments, listed in Table 1. TTO concentrations affected the amount of mold growth on white bread. Average CFU of bread mold dropped from 12 ± 0.6 and 17 ± 0.8 CFU on the negative controls to 0 CFU on bread treated with 0.06% and 0.24% TTO. Thus, tea tree oil inhibited the growth of mold.

To further verify the toxicity effect of TTO on eukaryotic cells, we hypothesized that 0.25% TTO will inhibit yeast growth which leads to decreased CO_2 production. The results of the experiment are shown in Figure 2. It was expected that as yeast used sugar as an energy source for fermentation, the balloons would increase in diameter from CO_2 production. The first column representing the positive control of the experiment showed that the diameter of the balloon increased by an average of 12 ± 3 cm, after approximately 40 minutes. The second column represents the results with the addition of 0.25% TTO, where the diameter of the balloon increased by an average of 6.67 ± 2.1 cm. Due to a small sample size (n=3), an unpaired Student's t-test was performed. Since p=0.07, the addition of 0.25% TTO did not significantly affect sugar utilization of *S. cerevisiae*, indicating TTO at 0.25% concentration is potentially non-toxic. The negative control bottles showed no gas production.

DISCUSSION

Tea Tree Oil is a potential natural alternative to household chemical cleaners that is still under-studied. This study tested the efficacy of TTO in reducing mold growth by using bread as a growth medium, and by evaluating its toxicity to eukaryotic cells by measuring the reduction of CO_2 production by *S. cerevisiae*.

In the first experiment, the bread sprayed with water had more mold colonies than just bread that was not sprayed. This was expected because previous research has shown that higher moisture content or humidity offers better growth conditions for mold (Axel et al., 2017). It has also been previously shown that TTO can inhibit grey mold Botrytis cinerea that forms on fruit, but the mechanism of how the growth is affected is still unclear (Shao et al., 2013). We found that 0.06% and 0.24% TTO concentrations inhibit visible colony formation at room temperature in humid and dark environments. Our results are similar to previously published data at these minimum inhibitory concentrations (MIC) against fungi (Hammer et al., 2002).

In the second experiment, it was found that 0.25% TTO did not significantly inhibit yeast growth as gas production in the treatment sample was not significantly different to those observed in the negative controls, suggesting a lack of toxicity at this concentration. Although, TTO has previously been shown to have antifungal effects against yeast such as Candida species (D'Auria et al., 2001). Given that the MIC values we used in the study were based on filamentous fungi and dermatophytes but not baker's yeast *S. cerevisiae* (Hammer et. al., 2002), further studies evaluating the toxicity effects of TTO using *S. cerevisiae* would help determine the safety limits of TTO.

Methods described in the study were used due to COVID-19 restrictions on in-person lab attendance, leading to a more 'citizen-science' approach. Thus, these results would need to be re-examined in a controlled laboratory setting to determine the effect of TTO on microbes.

There were some limitations in the experiment. 1) pH of media was not measured; it could not be ruled out as a factor affecting the growth of mold or yeast. 2) There could be volume inaccuracies between our replicates since our measuring techniques were not as precise as they would have been if we used calibrated pipettes. 3) We did not have access to various mold species, which would help establish the range of TTO concentrations required to inhibit them. In addition, future experiments can be performed to test the efficacy of TTO concentrations on surfaces using RODAC plate or the swab method. Another avenue of testing is to evaluate the microbial load with a microbial air sampler before and after spraying TTO.

CONCLUSIONS

In conclusion, it was found that TTO can inhibit visible common bread mold growth at the lowest concentration of 0.06%, but does not significantly reduce yeast viability even at 0.25%. This shows that tea tree oil can potentially be used as an antifungal in home settings, especially because it is a natural oil, and because it is efficient at low concentrations.

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REFERENCES

[1] Axel, C., Zannini, E., & Arendt, E. K. (2017). Mold spoilage of bread and its biopreservation: A review of current strategies for bread shelf life extension. *Critical Reviews in Food Science and Nutrition, 57*(16), 3528-3542. https://doi.org/10.1080/10408398.2016.1147417

[2] Cheung, I. M. Y., Xue, A. L., Kim, A., Ammundsen, K., Wang, M. T. M., & Craig, J. P. (2018). In vitro anti-demodectic effects and terpinen-4-ol content of commercial eyelid cleansers. *Contact Lens & Anterior Eye, 41(6), 513-517*. https://doi.org/10.1016/j.clae.2018.08.003

[3] Cox, S.D., Mann, C.M., Markam, J.L., Gustafson, J.E., Warmington, J.R., & Wyllie, S.G. (2001). Determining the antimicrobial actions of tea tree oil. *Molecules: A Journal of Synthetic Chemistry and Natural Product Chemistry, 6*(2), 87-91. https://doi.org/10.3390/60100087

[4] Dalwai, S., Rodrigues, S. J., Baliga, S., Shenoy, V. K., Shetty, T. B., Pai, U. Y., & Saldanha, S. (2016). Comparative evaluation of antifungal action of tea tree oil, chlorhexidine gluconate and fluconazole on heat polymerized acrylic denture base resin - an in

vitro study. *Gerodontology, 33*(3), 402-409. https://doi.org/10.1111/ger.12176

[5] D'Auria, F.D., Laino, L., Strippoli, V., Tecca, M., Salvatore, G., Battinelli, L., & Mazzanti, G. (2001). In vitro activity of tea tree oil against Candida albicans mycelial conversion and other pathogenic fungi. *Journal of chemotherapy, 13*(4), 377-383. https://doi.org/10.1179/joc.2001.13.4.377

[6] Garcia, M. V., & Copetti, M. V. (2019). Alternative methods for mould spoilage control in bread and bakery products. *International Food Research Journal, 26*(3), 737-749.

[7] Hammer, K. A., Carson, C. F., & Riley, T. V. (2002). In vitro activity of melaleuca alternifolia (tea tree) oil against dermatophytes and other filamentous fungi. *Journal of Antimicrobial Chemotherapy, 50*(2), 195-199. https://doi.org/10.1093/jac/dkf112

[8] Hammer, K. A., Carson, C. F., Riley, T. V., & Nielsen, J.B. (2006). A review of the toxicity of Melaleuca alternifolia (tea tree) oil. *Food & Chemical Toxicology, 44*(5), 616-625. https://doi.org/10.1016/j.fct.2005.09.001

[9] Low, W., Kenward, K., Britland, S. T., Amin, M. C., & Martin, C. (2017). Essential oils and metal ions as alternative antimicrobial agents: A focus on tea tree oil and silver. *International Wound Journal, 14*(2), 369-384. https://doi.org/10.1111/iwj.12611

[10] Melegari, G., Iseppi, R., Mariani, M., Guiliani, E., Caciagli, V., Bertellini, E., Messi, P. & Barbieri, A. (2021). Keyboard contamination in intensive care unit: is cleaning enough? Prospective research of in situ effectiveness of a tea tree oil (KTEO) film. *Advances in experimental medicine and biology, 1323*, 91.

[11] Reeds, P.J. (2000). Dispensable and indispensable amino acids for humans. *The Journal of Nutrition, 130*(7), 1835S–1840S. https://doi.org/10.1093/jn/130.7.1835S

[12] Rogawansamy, S., Gaskin, S., Taylor, M., & Pisaniello, D. (2015). An evaluation of antifungal agents for the treatment of fungal contamination in indoor air environments. *International Journal of Environmental Research and Public Health, 12*(6), 6319-6332. https://doi.org/10.3390/ijerph120606319

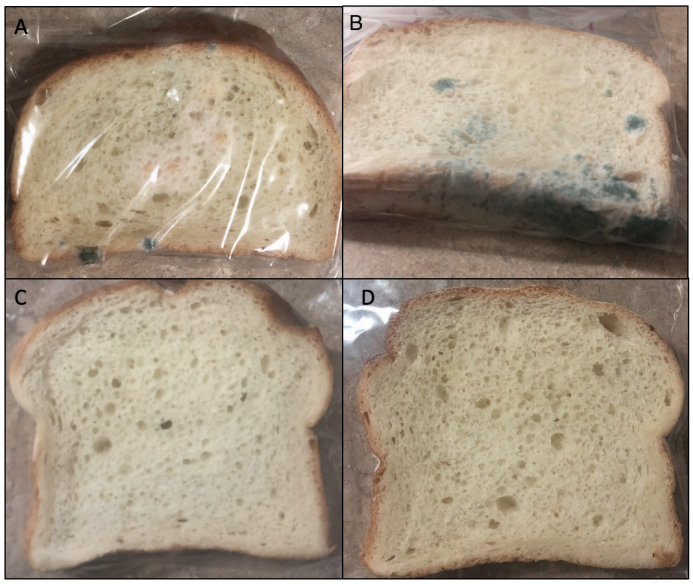
[13] Shao, X., Cheng, S., Wang, H., Yu, D., & Mungai, C. (2013). The possible mechanism of antifungal action of tea tree oil on Botrytis cinerea. *Journal of Applied Microbiology, 114*(6), 1642-1649. https://doi.org/10.1111/jam.12193

[14] Shi, C., Zhang, X. & Guo, N. (2018). The antimicrobial activities and action-mechanism of tea tree oil against food-borne bacteria in fresh cucumber juice. *Microbial Pathogenesis, 125*, 262-271. https://doi.org/10.1016/j.micpath.2018.09.036

[15] "*Tea tree oil*" National Center for Complementary and Integrative Health, US National Institutes of Health. November 9, 2011. Retrieved: April 6, 2021 from https://www.nccih.nih.gov/health/tea-tree-oil

[16] Vagelas, I., Gougoulias, N., Nedesca, E.D., & Liviu, G. (2011) Bread Contamination with Fungus. *Carpathian Journal of Food Science and Technology, 3*(2), 1-6.

[17] Zhang, Y., Hua, L., Liu, Q., Chu, S., Gan, Y., Wu, M., Bao, Y., Chen, Q., & Zhang, J. (2021). Household mold exposure interacts with inflammation-related genetic variants on childhood asthma: A case-control study. *BMC Pulmonary Medicine, 21*(1), 114-11. https://doi.org/10.1186/s12890-021-01484-9



Supplementary Figure 1 White bread from an additional trial exposed to different treatments after 7 days when placed in a dark, humid environment at ~25°C. A = no treatment; B = water; C = 0.06% TTO; D = 0.24% TTO.

Dataset for **Figure 2**. Diameter of balloons placed on the lip of the positive control and 0.25% TTO concentration bottles placed in a 45°C-water bath for 45 minutes.

Bottle contents	Balloon diameter (cm)				
	Trial 1		Trial 2	Average	STDEV
	Lovleen	Jenisha	Lovleen		
Positive control (100 mL water, 2g sugar, 2g yeast)	15	9	12	12	3
100 mL water, 2g sugar, 2g yeast, 0.25% TTO	9	6	5	6.6667	2.0817