MONASH UNIVERSITY

School of Applied Sciences

ESSA Report 1: Susceptibility tests - Solution studies 27/02/01

Background:

The minimum inhibitory concentration (MIC) of a chemical is the lowest concentration of the test agent that will inhibit the growth of the test bacteria i.e. it measures the bacteriostatic activity of the test agent.

The minimum bactericidal concentration (MBC) of a chemical is the lowest concentration of the test agent that will kill the test bacteria i.e. it measures the bacteriocidal activity of the test agent.

1. Susceptibility Testing of Bacteria Using a Standard Broth Dilution Method

Aim:
To determine the MIC and MBC of solutions of Tea Tree oil and Safe T Air gel on a wide range of airborne bacteria.

Method:
Susceptibility tests were carried out on a range of common airborne bacteria. Bacterial species were chosen to compare the results for Tea Tree oil and Safe T Air gel to previous published results (ESSA product information sheet). Cultures were obtained from a standard reference collection of bacteria (Australian Collection of Microorganisms).

Enteric bacteria (found in animal faeces)
Escherichia coli ATCC 25922
Klebsiella pneumoniae ATCC 13883
Proteus vulgaris ATCC 6280
Serratia marcescens NCTC 1377
Enterococcus faecalis ATCC 29212
Enterobacter aerogenes ATCC 13048
Pseudomonas putida ATCC 49128

Skin bacteria
Staphylococcus epidermidis ATCC 14990
Staphylococcus aureus NCTC 6571
Corynebacterium sp. ATCC 19409
Propionebacterium acnes ACM 821 (Australian Collection of Microorganisms)

Bacteria common in dust
Bacillus subtilis ATCC 11774
Bacillus cereus ATCC 10876A
Micrococcus luteus ATCC 10240

Environmental pathogens
Legionella pneumophila

Bacteria were tested against the following concentrations of tea tree oil/ Safe T Air gel:
1.0%, 0.75%, 0.5%, 0.4%, 0.3%, 0.2% and 0.1% mg/mL

The concentration of the Safe T Air gel was set up to obtain the same concentration of the oil component as the pure oil. Each organism was tested against all concentrations of the oil taken from both 100% pure tea tree

oil and from the Safe T Air gel using the macrodilution method described in the NCCLS reference. For each organism a full set of controls was also set up with equivalent amounts of 85% ethanol (used for dissolving both the pure oil and the gel) to those that would have been present in each of the dilutions. A control with neither tea tree oil nor ethanol was also included for each organism to check the viability of the inoculum.

Tea tree oil

MICs were determined by recording presence or absence of turbidity in the broth dilutions. After initial reading of the tubes the last three dilutions showing no apparent growth were plated out onto a suitable agar medium to determine the minimum bactericidal concentration (MBC).

Safe T Air gel

MICs of the gel-associated oil could not be read because the cloudiness of the diluted gel solutions made it impossible to read turbidity changes. MBCs were determined by plating out the diluted gel solutions.

Results:

In the table below G = growth, N = no growth and NAG = no apparent growth. No distinction has been made between levels of growth as this is difficult in broth cultures.

Organism	**Chemical**		**Concentration of tea tree oil/ Safe T Air gel (w/v%)**							
			1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Escherichia	100% oil	NAG	NAG	NAG	NAG	NAG	G	G	G	
coli	After plating	N	N	N	N	N	G	G		
	Ethanol only	G	G	G	G	G	G	G		
	Gel-assoc. oil	N	N	N	N	N	G	G		
	Ethanol only	NAG	NAG	G	G	G	G	G		

MIC = 0.3% MBC = 0.3%(oil), 0.3%(gel)

			1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Klebsiella	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	G	G	
pneumoniae	After plating	N	N	N	N	N	N	G		
	Ethanol only (oil dil. equiv.)	G	G	G	G	G	G	G		
	Gel-assoc. oil	N	N	N	N	N	N	G		
	Ethanol only (gel dil. equiv.)	NAG	NAG	G	G	G	G	G		

MIC = 0.2% MBC = 0.2%(oil), 0.2%(gel) Note that the gel-associated oil has a greater quantity of ethanol added with it, due to the low concentration of oil in the gel. This explains the difference between the results from the two sets of ethanol controls.

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Proteus	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	G	G
vulgaris	After plating	N	N	N	N	N	N	G	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	N	N	N	N	N	N	G	
	Ethanol only	NAG	G	G	G	G	G	G	

MIC = 0.2% MBC = 0.2%(oil), 0.2%(gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Serratia	100% oil	NAG	NAG	NAG	NAG	NAG	G	G	G
marcescens	After plating	N	N	N	N	N	G	G	
	Ethanol only	NAG	G	G	G	G	G	G	
	Gel-assoc. oil	N	N	G	G	G	G	G	
	Ethanol only	G	G	G	G	G	G	G	

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Enterococcus	100% oil	NAG	NAG	NAG	G	G	G	G	G
faecalis	After plating	N	G	G	G	G	G	G	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	N	N	G	G	G	N	G	
	Ethanol only	NAG	G	G	G	G	G	G	

MIC = 0.5% MBC = 1.0% (oil), 0.75%(gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Enterobacter	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	G	G
aerogenes	After plating	N	N	N	N	N	G	G	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	N	N	N	N	G	G	G	
	Ethanol only	NAG	NAG	G	G	G	G	G	

MIC = 0.2% MBC = 0.3% (oil), 0.4% (gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Pseudomonas	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
putida	After plating	N	N	N	N	N	N	G	
	Ethanol only	N	N	N	N	N	G	G	
	Gel-assoc. oil	N	N	N	N	N	N	G	
	Ethanol only	N	N	N	N	N	N	G	

MIC = 0.1% MBC = 0.2% (oil), 0.2% (gel) The gel results are inconclusive because the ethanol appears to be inhibiting growth at the same concentration.

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Staphylococcus	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
epidermidis	After plating	N	N	N	N	N	N	N	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	N	N	N	N	N	N	G	
	Ethanol only	NAG	G	G	G	G	G	G	

MIC = 0.1% MBC = 0.1 (oil), 0.2% (gel)

	1.0	0.75	0.5	0.4	0.3	0.2	0.1	0

Staphylococcus aureus	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
	After plating	N	N	N	N	N	N	N	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	N	N	N	N	N	N	G	
	Ethanol only	NAG	NAG	G	G	G	G	G	

MIC = 0.1% MBC = 0.1% (oil), 0.2% (gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Corynebact-erium sp.	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
	After plating	N	N	N	N	N	G	G	
	Ethanol only	NAG	NAG	G	G	G	G	G	
	Gel-assoc.oil	N	N	N	N	N	N	N	
	Ethanol only	NAG	NAG	NAG	NAG	G	G	G	

MIC = 0.3% MBC = 0.3%(oil), <0.1%(gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Propione-bacterium acnes	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
	After plating	N	N	N	N	N	N	G	
	Ethanol only	G	G	G	G	N	G	G	
	Gel-assoc. oil	N	N	N	N	N	N	N	
	Ethanol only	NAG	NAG	G	G	G	G	G	

MIC = 0.1% MBC = 0.2% (oil), <0.1%(gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Bacillus subtilis	100% oil	NAG	NAG	NAG	NAG	NAG	G	G	G
	After plating	G	G	G	G	G	G	G	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	N	G	G	G	G	G	G	
	Ethanol only	NAG	G	G	G	G	G	G	

MIC = 0.3% MBC = >1.0% (oil), >1.0% (gel)
MIC = 0.3% MBC = 0.3% (oil), 0.75%(gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Bacillus cereus	100% oil	NAG	NAG	NAG	NAG	?G	?G	G	G
	After plating	G	G	G	G	G	G	G	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	G	G	G	G	G	G	G	
	Ethanol only	G	G	G	G	G	G	G	

MIC = 0.2 - 0.4% MBC = >1.0% (oil), >1.0 % (gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Micrococcus	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G

luteus	After plating	N	N	N	N	N	N	N	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	N	N	N	N	N	N	G	
	Ethanol only	G	G	G	G	G	G	G	

MIC = 0.1% MBC = 0.1% (oil), 0.2% (gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Legionella	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
pneumophila	After plating	N	N	N	N	N	N	N	
	Ethanol only	N	N	G	G	G	G	G	
	Gel-assoc. oil	N	N	N	N	N	N	N	
	Ethanol only	N	N	N	N	G	G	G	

MIC = <0.1% MBC = <0.1% (oil), <0.1% (gel)

Summary:

Bacteria	**Minimum inhibitory concentration (MIC) % mg/mL**		**Minimum bactericidal concentration (MBC) % mg/mL**	
	Tea tree oil	**Safe T Air gel#**	**Tea tree oil**	**Safe T Air gel#**
Escherichia coli	0.3	*	0.3	0.3
Klebsiella pneumoniae®	0.2	*	0.2	0.2
Proteus vulgaris®	0.2	*	0.2	0.2
Enterococcus faecalis	0.5	*	1.0	0.75
Serratia marcescens®	0.3	*	0.3	0.75
Enterobacter aerogenes®	0.2	*	0.3	0.4
Pseudomonas putida®	0.1	*	0.2	~
Staphylococcus epidermidis	0.1	*	0.1	0.2
Staphylococcus aureus	0.1	*	0.1	0.2
Corynebacterium sp.	0.1	*	0.3	<0.1
Propionebacterium acnes	0.1	*	0.2	<0.1
Bacillus subtilis	0.3	*	>1.0	>1.0
Bacillus cereus	0.2	*	>1.0	>1.0
Micrococcus luteus	0.1	*	0.1	0.2

~ inconclusive result # concentration of oil in the gel solution

* not readable due to cloudiness of gel solution ® reproduced in duplicate

Conclusion:

Minimum Inhibitory Concentration (MIC)

The growth of all bacteria tested was inhibited by tea tree oil at concentrations varying from 0.1 to 0.5%.

Minimum Bactericidal Concentration (MBC)

Both tea tree oil and Safe T Air gel were shown to have a bactericidal effect on most test bacteria at concentrations of 0.1 to 0.4%. The results for tea tree oil confirm earlier studies published by ESSA.

Three species were shown be more resistant than the others:

Enterococcus faecalis	MBC (oil) 1.0% MBC (gel) 0.75%
Bacillus subtilis	MBC (oil) >1.0% MBC (gel) >1.0%
Bacillus cereus	MBC (oil) >1.0% MBC (gel) >1.0%

The resistance of *Bacillus subtilis* and *Bacillus cereus* is probably related to their ability to form resistant endospores.

2. Susceptibility Testing of Fungi Using a Standard Broth Dilution Method

Aim:

To determine the MIC and MFC of solutions of Tea Tree Oil on a wide range of airborne yeasts and moulds.

Test fungi:
Cultures were obtained from a standard reference collection of fungi (Australian Collection of Microorganisms).

Yeasts	*Candida albicans*	ATCC 2091
Moulds	*Trichophyton mentagrophytes*	ATCC 9533
	Trichophyton rubrum	ATCC 28188
	Aspergillus niger	ATCC 6275
	Aspergillus flavus	ATCC 15546

Each organism was tested against all concentrations of the oil taken from 100% pure tea tree oil using a modified macrodilution method described by Arzeni et al(1998) and McGinnis & Pasarell (1998). For each organism a full set of controls was also set up with equivalent amounts of 85% ethanol to those that would have been present in each of the dilutions. A control with neither tea tree oil nor ethanol was also included for each organism to check the viability of the inoculum.

After initial reading of the tubes the last three dilutions showing no apparent growth were plated out onto a suitable agar medium to distinguish between the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC).

The tests with the gel dilutions could not be performed due to clouding of the broth caused by the gel.

Results:

In the table below G = growth, N = no growth and NAG = no apparent growth. No distinction has been made between levels of growth, as this is difficult in broth cultures.

Organism	**Chemical**	**Concentration of tea tree oil (w/v%)**							
		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Candida	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	G	G
albicans	After plating	N	N	N	N	G	G	G	
	Ethanol only	G	G	G	G	G	G	G	
MIC = 0.2%	MFC = 0.4%								
		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Aspergillus	100% oil	NAG	NAG	NAG	G	G	G	G	G
flavus	After plating	N	N	G	G	G	G	G	
	Ethanol only	G	G	G	G	G	G	G	
MIC = 0.5%	MFC = 0.75%								
		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Aspergillus	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
niger	After plating	N	N	G	G	G	G	G	
	Ethanol only	G	G	G	G	G	G	G	
MIC = 0.1%	MFC = 0.75%								

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Trichophyton	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	G	G
mentagrophytes	After plating	N	N	N	N	G	G		
	Ethanol only	G	G	G	G	G	G	G	

MIC = 0.2% MFC = 0.4%

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
Trichophyton	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
rubrum	After plating	N	N	N	N	N	N	G	
	Ethanol only	NAG	G	G	G	G	G	G	

MIC = 0.1% MFC = 0.2%

Summary:

Fungi	**Minimum inhibitory concentration (MIC) % mg/mL** **Tea tree oil**	**Minimum fungicidal concentration (MBC) % mg/mL** **Tea tree oil**
Candida albicans	0.2	0.4
Aspergillus flavus	0.5	0.75
Aspergillus niger	0.1	0.75
Trichophyton mentagrophytes	0.2	0.4
Trichphyton rubrum	0.1	0.2

Conclusion:

The MIC of the chemical is the lowest concentration of the test agent that will inhibit the growth of the test fungi i.e. it measures the fungiostatic activity of the test agent. The growth of all fungi tested was inhibited by tea tree oil at concentrations varying from 0.1 to 0.5%.

The MFC of the chemical is the lowest concentration of the test agent that will kill the test fungi i.e. it measures the fungicidal activity of the test agent. Tea tree oil was shown to have a fungicidal effect on the test fungi at concentrations of 0.2 to 0.75%.

References:

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27/02/01