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Dear John

MICROBIOLOGICAL TESTS WITH DUCANE KUNZEA OIL (Kunzea ambigua)
(SUMMARY)

Over the last year or so, several ad hoc trials have been conducted to obtain some practical information on the effect of kunzea oil on a few selected microorganisms. There were 2 basic types of test: (1) qualitative tests on the effect of kunzea oil on bacteria and moulds applied to a surface (in this case, organisms from a liquid suspension were swabbed onto squares of sterilised vinyl floor covering), and (2) semi-quantitative or quantitative estimations of microbial populations in suspensions containing various concentrations of kunzea oil, in most cases over a period of time - a non-toxic surface active agent was usually added to keep the oil dispersed, and other materials were also added in a few cases.

15.03.01
Test organism/s: Aspergillus flavus, A. fumigatus, Alternaria alternata
Test type: liquid suspension
Dispersant: Tween 40 approx 1 %

Viability after 5 days (growth from swab spread on Sabouraud Agar)

| KO concn. | 1% | 10% | 20% |
|---|---|---|---|
| Asp. flavus | + | + | + |
| Asp. fumigatus | + | + | - |
| Alt. alternata | + | - | - |

+= growth; - = no growth

15.03.01
Test organism/s: Staph aureus
Test type: liquid
Dispersant: Tween 40 approx 1 %

| KO concn. / Growth from subculture after | 1% | 10% | 20% |
|---|---|---|---|
| 30 min | ++ | + | ± |
| 24 h | - | - | - |

++= profuse growth; ±= limited growth; -= no growth

17.07.01
Test organism/s: Staph (MRSA), Aspergillus flavus, A. fumigatus, Alternaria alternata
Test type: vinyl floor tile surface
Additive:

Growth from swabbed suface

| Organism | Time interval | KO 0.1% | KO 0.5% | KO 1.0% | KO 2.0% | KO 5.0% | KO 10.0% |
|---|---|---|---|---|---|---|---|
| Staph | 15min | + | + | + | + | + | + |
| " | 1h | + | + | + | + | + | ± |
| " | 24h | + | + | ± | - | - | - |
| Aspergillus flavus | 1h | + | + | + | + | + | + |
| " | 16h | + | + | + | + | ± | ± |
| Asp. fumigatus | 1h | + | + | + | + | + | + |
| " | 16h | + | + | + | + | + | ± |
| Alternaria alternata | 1h | + | + | + | + | + | + |
| " | 16h | + | + | + | + | + | ± |
| Legionella pneumophila | 1h | + | + | + | + | + | + |
| " | 16h | + | + | + | + | + | ± |

++= profuse growth; ±= limited growth; -= no growth

20.09.01
Test organism/s: Staph aureus combined with Aspergillus flavus
Test type: vinyl floor tile surface
Additive: Tergitol 7 (note: this compound has an inherent inhibitory effect on some types of bacteria)

| | | Recovery/ growth from swab at 24h* | | |
|---|---|---|---|---|
| KO % | Tergitol 7 % | 2.5 | 5.0 | 10.0 |
| 0 | | - | + | + |
| 0.2 | | - | - | + |
| 0.35 | | + | + | - |
| 0.5 | | + | - | - |

* all + after 1h only

29.09.01
Test organism/s: Staph aureus
Test type: vinyl floor tile surface
Additive: Tergitol 7

| KO % | 0 | | | 0.2 | | | 0.35 | | | 0.5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| At time (hr) | <1 | 24 | 48 | <1 | 24 | 48 | <1 | 24 | 48 | <1 | 24 | 48 |
| T7 2.5% | ++ | + | ± | ++ | ± | ± | ++ | - | - | + | ± | - |
| T7 5.0% | ++ | + | ± | ++ | ± | ± | ++ | ± | - | + | ± | ± |
| T7 10% | + | ± | - | + | - | ± | ++ | + | - | + | ± | - |

30.09.01
Test organism/s: Staph aureus
Test type: vinyl floor tile surface
Additive: Tergitol 7

| KO % | 0 | | | 0.2 | | | 0.35 | | | 0.5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| At time (hr) | <1 | 24 | 48 | <1 | 24 | 48 | <1 | 24 | 48 | <1 | 24 | 48 |
| T7 2.5% | ++ | + | ± | ++ | ± | ± | ++ | - | - | nt | ± | - |
| T7 5.0% | ++ | + | ± | ++ | ± | ± | ++ | ± | - | nt | ± | ± |
| T7 10% | + | ± | - | + | - | ± | ++ | ± | - | nt | ± | - |

NB: ++= profuse growth; -= no growth; ± = <10 colonies; nt= not tested

27.10.01
Test organism/s: Staph (MRSA)
Test type: vinyl floor tile surface
Additive: proprietary green gel (unknown composition)

Culture applied to surface before oil applied

| KO concn. | 0 (gel only) | 0.2% | 0.35% | 0.5% | nil (control) | oil only |
|---|---|---|---|---|---|---|
| 1h | + | + | + | + | + | + |
| 24h | + (100*) | + (100) | + (60) | + (60) | ++(>>100) | + |

Culture applied to surface after oil applied

| KO concn. | 0 (gel only) | 0.2% | 0.35% | 0.5% | nil (control) | oil only |
|---|---|---|---|---|---|---|
| 1h | + | + | + | + | + | + |
| 24h | + (10) | + (50) | + (20) | + (8) | ++(>>100) | + |

++= profuse growth; ±= limited growth; -= no growth; * approx no. of colonies

24.11.01
Test organism/s: Staph aureus
Test type: vinyl floor tile surface (bacteria applied before treatment with oil)
Additive:

Growth from surface swab

| KO % / At time (hr) | 0 | 5% | 10% | undiluted |
|---|---|---|---|---|
| 15 min | ++ | ++ | + | + |
| 1 hr | ++ | ++ | + | + |
| 2 hr | ++ | + | + | ± |
| 3 hr | + | + | ± | ± |
| 6 hr | ± | ± | - | - |
| 14 hr | ± | ± | ± | - |
| 15 hr | ± | ± | - | - |

++= profuse growth; ±= limited growth; -= no growth

14.12.01
Test organism/s: Staph (MRSA)
Test type: vinyl floor tile surface
Additive: grapeseed oil

Recovery (relative growth from swab spread)

| Treatment / Time | grapeseed oil only | K O only | KO + grapeseed oil 1:1 | Tween 80 (0.1%) only | Tween 80 +5% KO | Tween 80 + 10% KO | Tween 80 +20% KO |
|---|---|---|---|---|---|---|---|
| 0 | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| 1h | + | ± | - | ++ | + | + | - |
| 4h | + | - | - | ++ | - | - | - |
| 24h | - | - | - | ++ | - | - | - |

++= profuse growth; ±= limited growth; -= no growth

23.12.01
Test organism/s: Staph (MRSA)
Test type: liquid suspension
Additive: grapeseed oil, Tween 80

Plate counts after addition of culture

| Treatment / Time | grapeseed oil only | K O only | KO + grapeseed oil I:I | Tween 80 (0.1%) only | Tween 80 +5% KO | Tween 80 + 10% KO | Tween 80 +20% KO |
|---|---|---|---|---|---|---|---|
| 1h | 93 000 | 2 600 | 3 900 | 17 000 | 0 | 0 | 0 |
| 7h | 40 000 | 2 600 | 0 | 19 000 | 0 | 0 | 0 |
| 24h | 19 000 | not recorded | 0 | 7 700+ | 0 | 0 | 0 |

14.02.02
Test organism/s: Staph aureus NCTC 6571
Test type: liquid suspension
Additive: 0.1% Tween 80

Pour plate counts

| Count per plate after | nil treatment | KO 5% |
|---|---|---|
| <15 min | ~12 000 | 730 |
| 45 min | ~10 000 | 3 |
| 18 hr | ~11 000 | 0 |

14.02.02

Test organism/s:Legionella pneumophila
Test type: liquid suspension
Additive: 0.1% Tween 80

Spread plate

| Count per plate after | nil treatment | KO 5% |
|---|---|---|
| <15 min | ++ | ++ |
| 45 min | ++ | - |
| 18 hr | 2 cfu | ± (1 cfu) |

++= profuse growth; ±= limited growth; -= no growth

30.03.02
Aspergillus flavus showed no change with the same treatment conditions as 14.03.02

12.05.02
Test organism/s: Staph aureus (MRSA)
Test type: liquid suspension
Additive: 0.1% Tween 80

Colony counts (cfu) (surface spread method)

| Count per plate after | nil treatment | KO 0.1% | KO 0.2% | KO 0.3% | KO 0.4% |
|---|---|---|---|---|---|
| <15 min | 65 | 34 | 51 | 38 | 36 |
| 15 min | 55 | 25 | 22 | 19 | 9 |

30.06.02
Test organism/s: Staph aureus (MRSA)
Test type: liquid suspension
Additive: 0.1% Tween 80

Membrane filter counts (cfu)

| Count per plate after | nil | KO 1% | KO 2% | KO 3% | KO 4% | KO 5% |
|---|---|---|---|---|---|---|
| <15 min | 1500 | * | * | * | * | * |
| 1 hr | 3000 | 24 | 21 | 16 | 4 | 12 |
| 24 hr | 20 | 0 | 0 | 1 | 2 | 0 |

* initial counts omitted

13.07.02
Test organism/s: Staph aureus (MRSA)
Test type: liquid suspension
Additive: 0.1% Tween 80 + buffered peptone water 0.5%

Plate counts (pour plate method)

| Time | No. of cfu* | | Relative count (4% KO count÷untreated count) |
|---|---|---|---|
| | control | 4% KO | |
| start (<15min) | 130 | 110 | 85 |
| 2 hr | 93 | 104 | 110 |
| 18 hr | 1300 | 400 | 31 |
| 30 hr | 93 | 19 | 20 |
| 60 hr | 320 | 0 | <0.3 |

*cfu = colony-forming units ("colonies")

08.08.02
Test organism/s: Legionella pneumophila
Test type: liquid suspension
Additive: 0.1% Tween 80 + buffered peptone water 0.5% (pH 7.15)

| Time after addition of culture | Colony count after addition of bacterial suspension | | | |
|---|---|---|---|---|
| | Kunzea oil % | | | |
| | nil | 1.0 | 2.0 | 4.0 |
| 15 min | ~100 | 0 | 0 | 0 |
| 1h | 50 | 0 | 0 | 0 |
| 3h | 10 | 0 | 0 | 0 |
| 4h | 10 | 0 | 0 | 0 |
| 5h | 3 | 0 | 0 | 0 |

GENERAL COMMENTS: The above mini-trials were conducted in a variety of ways, and have indicated the antibacterial effect of kunzea oil in-vitro. Kunzea oil appeared to be effective against both staphylococci and legionella, with a reduction in counts over time, as compared with controls. A recent test (08.08.02) suggested that kunzea oil was particularly inhibitory towards legionella, with counts showing a significant reduction in a relatively short time (this was also suggested in a previous exploratory test)

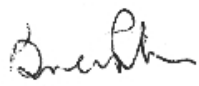
The effect of kunzea oil was perhaps less distinct in trials with cultures applied to surfaces, although positive trends were seen in some trials (note that additives other than a dispersant were included in some of the earlier surface trials).

Kunzea oil appeared to have only a limited effect on the 3 fungal species tested. This was the general result each time any of the trials were repeated, and significantly higher concentrations were needed to have any noticeable inhibitory effect under the trial conditions.

After some trial and error, Tween 80 was adopted as the standard non-inhibitory surface active agent to keep the kunzea oil in suspension with these trials. Tween 80 is a non-inhibitory surface-active agent that is commonly used in media and, more particularly, in solutions used in recovering microorganisms from surfaces etc.

In the recent tests of 13.07.02 and 08.08.02, a small amount of buffered peptone was added to the suspension to (a) maintain pH close to neutral, thereby reducing any potential inhibitory effect on organisms due to additives that have a sub-optimal pH (mostly acidic), and (b) to provide some easily-utilised nutrient so that any decrease in the number of organisms over time would not simply be as a result of starvation. The expectation was that a small amount of added peptone might reduce the rate of decline in population and possibly provide sufficient nutrient to support a limited amount of bacterial growth - although there is some variation (probably including sampling error) in the counts of the control group during the trial of 13.07.02, the counts were of a similar order and this made it easier to relate to the added effect of kunzea oil.

yours sincerely



Bruce Pike