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(54) **Title:** MICROBICIDAL COMPOSITION

(57) **Abstract:** A synergistic microbicidal composition comprising: (a) 4,4-dimethyloxazolidine; and (b) pine oil.



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MICROBICIDAL COMPOSITION

This invention relates to microbicidal compositions containing 4,4-dimethyloxazolidine and pine oil.

5 Pine extracts are known to have bactericidal effects, as disclosed, e.g., in GB2354771. However, this reference does not suggest the particular combination claimed herein, or the weight ratios, or suggest that any combination might be synergistic. The problem addressed by this invention is to provide additional synergistic combinations of microbicides.

10 STATEMENT OF THE INVENTION

The present invention is directed to a synergistic microbicidal composition comprising: (a) 4,4-dimethyloxazolidine; and (b) pine oil.

DETAILED DESCRIPTION OF THE INVENTION

15 As used herein, the following terms have the designated definitions, unless the context clearly indicates otherwise. The term "microbicide" refers to a compound capable of inhibiting the growth of or controlling the growth of microorganisms; microbicides include bactericides, fungicides and algacides. The term "microorganism" includes, for example, fungi (such as yeast and mold), bacteria, archaea and algae. "DMO" is 4,4-
20 dimethyloxazolidine. Unless otherwise specified, temperatures are in degrees centigrade (°C), references to percentages are percentages by weight (wt%) and amounts and ratios are on an active ingredient basis. Unless otherwise specified, all operations were performed at room temperature (20-25 °C).

Preferably, a weight ratio of 4,4-dimethyloxazolidine to pine oil is from 1:0.01 to
25 1:0.13 or 1:0.15 to 1:0.25 or 1:0.60 to 1:2.00 or 1:20 to 1:480. Preferably, the weight ratio of DMO to pine oil is from 1:0.01 to 1:0.09 or 1:0.15 to 1:0.25 or 1:0.60 to 1:1.60 or 1:20 to 1:480.

Preferably, the synergistic microbicidal compositions is substantially free of microbicides other than DMO and pine oil, i.e., it has less than 1 wt% of other microbicides
30 based on total weight of active ingredients, preferably less than 0.5 wt%, preferably less than 0.2 wt%, preferably less than 0.1 wt%. Preferably, when a synergistic microbicidal composition is added to an aqueous medium, the medium is substantially free of other microbicides, i.e., it has less than 1 wt% of other microbicides based on total weight of active

ingredients, preferably less than 0.5 wt%, preferably less than 0.2 wt%, preferably less than 0.1 wt%.

The compositions of this invention may contain other ingredients, e.g., defoamers and emulsifiers. The microbicidal compositions of the present invention can be used to inhibit the growth of microorganisms or higher forms of aquatic life (such as protozoans, invertebrates, bryozoans, dinoflagellates, crustaceans, mollusks, etc) by introducing a microbicidally effective amount of the compositions into an aqueous medium subject to microbial attack. Suitable aqueous media are found in, for example: industrial process water; electrocoat deposition systems; cooling towers; air washers; gas scrubbers; mineral slurries; wastewater treatment; ornamental fountains; reverse osmosis filtration; ultrafiltration; ballast water; evaporative condensers; heat exchangers; pulp and paper processing fluids and additives; starch; plastics; emulsions; dispersions; paints; latices; coatings, such as varnishes; construction products, such as mastics, caulks, and sealants; construction adhesives, such as ceramic adhesives, carpet backing adhesives, and laminating adhesives; industrial or consumer adhesives; photographic chemicals; printing fluids; household products, such as bathroom and kitchen cleaners; cosmetics; toiletries; shampoos; soaps; personal care products such as wipes, lotions, sunscreen, conditioners, creams, and other leave-on applications; detergents; industrial cleaners; floor polishes; laundry rinse water; metalworking fluids; conveyor lubricants; hydraulic fluids; leather and leather products; textiles; textile products; wood and wood products, such as plywood, chipboard, flakeboard, laminated beams, oriented strandboard, hardboard, and particleboard; petroleum processing fluids; fuel; oilfield fluids, such as injection water, fracture fluids, and drilling muds; agriculture adjuvant preservation; surfactant preservation; medical devices; diagnostic reagent preservation; food preservation, such as plastic or paper food wrap; food, beverage, and industrial process pasteurizers; toilet bowls; recreational water; pools; and spas.

The present invention is further directed to a method for inhibiting microbial growth in a medium by adding to the medium a synergistic composition as described herein. In a preferred embodiment, the synergistic composition is added to a medium susceptible to growth of fungi such as *A. brasiliensis*; wherein the weight ratio of DMO to pine oil is 1:20 to 1:480. In a preferred embodiment, the synergistic composition is added to a medium susceptible to growth of yeast such as *C. albicans*; wherein the weight ratio of DMO to pine oil is 1:0.01 to 1:0.13 or 1:0.15 to 1:0.25. In a preferred embodiment, the synergistic composition is added to a medium susceptible to growth of gram positive bacteria such as *S. aureus*; wherein the weight ratio of DMO to pine oil is 1:0.60 to 1:2.00.

The specific amount of the microbicidal composition of this invention necessary to inhibit or control the growth of microorganisms in an application will vary. Typically, the amount of the composition of the present invention is sufficient to control the growth of microorganisms if it provides from 50 to 5,000 ppm (mg/L) active ingredients of the composition. It is preferred that the active ingredients be present in the medium to be treated in an amount of at least 100 ppm, preferably at least 200 ppm, preferably at least 300 ppm. It is preferred that the active ingredients of the composition be present in the medium to be treated in an amount of no more than 4,000 ppm, preferably no more than 3,000 ppm, preferably no more than 2,000 ppm, preferably no more than 1,000 ppm, preferably no more than 800 ppm, preferably no more than 700 ppm. In a method of this invention, a composition is treated to inhibit microbial growth by adding, together or separately, the two components of the synergistic composition, in amounts that would produce the concentrations indicated above.

EXAMPLES

DMO with Pine oil was evaluated for synergy by determining the synergy index (S.I.) of the combination.. Incubation temperature for bacteria was 30°C and for fungi was 25°C. Synergy index was calculated based on minimum inhibitory concentrations (MIC) of two antimicrobial compounds (CA and CB) alone and in combinations (Ca and Cb). The test organisms were gram negative bacteria (*Pseudomonas aeruginosa* ATCC # 15442), gram positive bacteria (*Staphylococcus aureus* ATCC # 6538), and yeast (*Candida albicans* ATCC #10231) and mold (*Aspergillus brasiliensis* ATCC# 16404). The test was carried out in 96 well microtiter plates. Contact time for the bacteria and yeast was 24 and 48 hours, and contact time for mold was 3 and 7 days.

The test results for demonstration of synergy are shown in Table 4, which shows the combinations of two components, Synergy Index against the microorganisms tested with incubation times; the end-point activity in ppm measured by the MIC for compound A alone (CA), for component B alone (CB), and the mixture (Ca) and (Cb); the calculated SI value; and the range of synergistic ratios for each combination tested. SI is calculated as follows:

$$Ca/CA + Cb/CB = \text{Synergy Index ("SI")}$$

Wherein:

CA = concentration of compound A in ppm, acting alone, which produced an end point (MIC of Compound A).

Ca = concentration of compound A in ppm, in the mixture, which produced an end point.

CB = concentration of compound B in ppm, acting alone, which produced an end point (MIC of Compound B).

Cb = concentration of compound B in ppm, in the mixture, which produced an end point.

When the sum of Ca/CA and Cb/CB is greater than one, antagonism is indicated. When the sum is equal to one, additivity is indicated, and when less than one, synergism is demonstrated.

Table1 Inoculums & concentration in each well (CFU/ml) used for synergy test

<i>Staphylococcus aureus</i> ATCC# 6538	<i>Pseudomonas aeruginosa</i> ATCC # 15442	<i>Aspergillus brasilienses</i> ATCC# 16404	<i>Candida albicans</i> ATCC#10231
3.108E+06	1.808E+05	4.032E+04	8.134E+04

Table2 Media Used for Synergy test

<i>Staphylococcus aureus</i> ATCC# 6538	<i>Pseudomonas aeruginosa</i> ATCC # 15442	<i>Aspergillus brasilienses</i> ATCC# 16404	<i>Candida albicans</i> ATCC#10231
10% Tryptic soy broth	10% Tryptic soy broth	Potato dextrose broth	Potato dextrose broth

Table 3 Compounds used for testing

Sample #	Product	CAS#	AI %	Solvent used to prepare stocks
1	DMO	51200-87-4	78%	H ₂ O
2	Pine oil	8002-09-3	100%	DMSO

5

The source of pine oil was Swastik Chemicals in India.

Table4

Ca:component in ppm AI of DMO (4,4- dimethyl oxazolidine)

Cb:component in ppm of Pine Oil

Ratio:Ca:Cb

Test Organisms	Contact Time	Ca	Cb	S.I.	Ca:Cb
<i>A.brasiliensis</i> ATCC # 16404	7 days	500	0	-	-
		0	8000	-	-
		400	3000	1.18	1:7.50
		200	4000	0.90	1:20.00
		100	5000	0.83	1:50.00
		50	6000	0.85	1:120.00
		25	6000	0.80	1:240.00
		12.5	6000	0.78	1:480.00
<i>C.albicans</i> ATCC # 10231	24 hrs	1000	-	-	-
		-	300	-	-
		700	8	0.73	1:0.01
		700	10	0.73	1:0.01
		700	20	0.77	1:0.03
		700	30	0.80	1:0.04
		700	40	0.83	1:0.06
		700	50	0.87	1:0.07
		700	60	0.90	1:0.09
		700	80	0.97	1:0.11
		700	100	1.03	1:0.14
		500	30	0.60	1:0.06
		500	40	0.63	1:0.08
		500	50	0.67	1:0.10
		500	60	0.70	1:0.12
		500	80	0.77	1:0.16
		500	100	0.83	1:0.20
		500	200	1.17	1:0.40
		400	50	0.57	1:0.13
		400	60	0.60	1:0.15
		400	80	0.67	1:0.20
		400	100	0.73	1:0.25
		400	200	1.07	1:0.50
	48 hrs	1000	-	-	-
		-	400	-	-
		700	50	0.83	1:0.07
		700	60	0.85	1:0.09
		700	70	0.88	1:0.10
		700	80	0.90	1:0.11
		700	90	0.93	1:0.13

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		700	100	0.95	1:0.14
		700	200	1.20	1:0.29
		500	50	0.63	1:0.10
		500	60	0.65	1:0.12
		500	70	0.68	1:0.14
		500	80	0.70	1:0.16
		500	90	0.73	1:0.18
		500	100	0.75	1:0.20
		500	200	1.00	1:0.40
		400	200	0.90	1:0.50
		400	300	1.15	1:0.75
		200	300	0.95	1:1.50
<i>S.aureus</i> ATCC#6538	24hrs	75	-	-	-
		-	400	-	-
		50	30	0.74	1:0.60
		50	40	0.77	1:0.80
		50	50	0.79	1:1.00
		50	60	0.82	1:1.20
		50	80	0.87	1:1.60
		50	100	0.92	1:2.00
	48hrs	50	200	1.17	1:4.00
		75	-	-	-
		-	400	-	-
		50	50	0.79	1:1.00
		50	60	0.82	1:1.20
		50	80	0.87	1:1.60
		50	100	0.92	1:2.00
		50	200	1.17	1:4.00

CLAIMS

1. A synergistic microbicidal composition comprising: (a) 4,4-dimethyloxazolidine; and (b) pine oil.
2. The synergistic microbicidal composition of claim 1 wherein a weight ratio of 4,4-dimethyloxazolidine to pine oil is from 1:0.01 to 1:0.13 or 1:0.15 to 1:0.25 or 1:0.60 to 1:2.00 or 1:20 to 1:480.
3. A method for inhibiting microbial growth in a medium by adding to the medium a synergistic microbicidal composition comprising: (a) 4,4-dimethyloxazolidine; and (b) pine oil.
4. The method of claim 3 wherein a weight ratio of 4,4-dimethyloxazolidine to pine oil is from 1:0.01 to 1:0.13 or 1:0.15 to 1:0.25 or 1:0.60 to 1:2.00 or 1:20 to 1:480.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/038598

A. CLASSIFICATION OF SUBJECT MATTER

INV. A01N43/76 A01N65/06
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2012/004272 A1 (JI KATHY J [CN] ET AL) 5 January 2012 (2012-01-05)	1,3
A	abstract; claims; examples; tables -----	2,4
Y	CA 2 722 533 A1 (ROHM & HAAS [US]) 18 January 2009 (2009-01-18)	1,3
A	page 1 - page 4; claims; examples ----- -/--	2,4



Further documents are listed in the continuation of Box C.



See patent family annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	C. T. D. PRICE ET AL: "Pine Oil Cleaner-Resistant Staphylococcus aureus: Reduced Susceptibility to Vancomycin and Oxacillin and Involvement of SigB", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 68, no. 11, 1 November 2002 (2002-11-01), pages 5417-5421, XP055107453, ISSN: 0099-2240, DOI: 10.1128/AEM.68.11.5417-5421.2002	1,3
A	discussion; page 5419 - page 5420 -----	2,4

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2016/038598

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2012004272	A1	05-01-2012	BR PI0923974 A2 11-08-2015
		CN 102361553 A 22-02-2012	
		JP 5536867 B2 02-07-2014	
		JP 2012521368 A 13-09-2012	
		US 2012004272 A1 05-01-2012	
		WO 2010108324 A1 30-09-2010	

CA 2722533	A1	18-01-2009	AU 2008203011 A1 05-02-2009
		CA 2637084 A1 18-01-2009	
		CA 2722085 A1 18-01-2009	
		CA 2722097 A1 18-01-2009	
		CA 2722117 A1 18-01-2009	
		CA 2722123 A1 18-01-2009	
		CA 2722200 A1 18-01-2009	
		CA 2722343 A1 18-01-2009	
		CA 2722533 A1 18-01-2009	
		CN 101347118 A 21-01-2009	
		CN 102246769 A 23-11-2011	
		CN 102273463 A 14-12-2011	
		CN 102302019 A 04-01-2012	
		CN 102308848 A 11-01-2012	
		CN 102318614 A 18-01-2012	
		CN 102318615 A 18-01-2012	
		CN 102318616 A 18-01-2012	
		EP 2025228 A2 18-02-2009	
		EP 2119354 A1 18-11-2009	
		EP 2119355 A2 18-11-2009	
		EP 2119356 A2 18-11-2009	
		EP 2119357 A2 18-11-2009	
		EP 2119358 A2 18-11-2009	
		EP 2119359 A2 18-11-2009	
		EP 2119360 A2 18-11-2009	
		JP 4944843 B2 06-06-2012	
		JP 5356467 B2 04-12-2013	
		JP 5356468 B2 04-12-2013	
		JP 5367025 B2 11-12-2013	
		JP 5367026 B2 11-12-2013	
		JP 5367027 B2 11-12-2013	
		JP 5367028 B2 11-12-2013	
		JP 5367029 B2 11-12-2013	
		JP 2009024003 A 05-02-2009	
		JP 2011251995 A 15-12-2011	
		JP 2011251996 A 15-12-2011	
		JP 2011251997 A 15-12-2011	
		JP 2011251998 A 15-12-2011	
		JP 2011251999 A 15-12-2011	
		JP 2011252000 A 15-12-2011	
		JP 2011252001 A 15-12-2011	
		JP 2011256194 A 22-12-2011	
		KR 20090009116 A 22-01-2009	
		KR 20100124694 A 29-11-2010	
		KR 20100124695 A 29-11-2010	
		KR 20100125207 A 30-11-2010	
		KR 20100125208 A 30-11-2010	
		KR 20100125209 A 30-11-2010	
		KR 20100126640 A 02-12-2010	
		KR 20100126641 A 02-12-2010	
		TW 200904333 A 01-02-2009	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2016/038598

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		TW 201216852 A	01-05-2012
		TW 201218947 A	16-05-2012
		TW 201218948 A	16-05-2012
		TW 201218949 A	16-05-2012
		TW 201218952 A	16-05-2012
		TW 201218953 A	16-05-2012
		TW 201218954 A	16-05-2012
		US 2009023688 A1	22-01-2009
		ZA 200806096 B	29-07-2009
