(12) STANDARD PATENT (11) Application No. **AU 2019422223 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Treatment of plants against disease.

(51) International Patent Classification(s)
A01N 65/00 (2009.01) ***A01N 65/40*** (2009.01)
A01N 25/02 (2006.01) ***A01P 1/00*** (2006.01)
A01N 59/00 (2006.01)

(21) Application No: **2019422223** (22) Date of Filing: **2019.09.16**

(87) WIPO No: **WO20/149749**

(30) Priority Data

(31) Number	(32) Date	(33) Country
749953	**2019.01.14**	**NZ**
755033	**2019.07.01**	**NZ**

(43) Publication Date: **2020.07.23**
(44) Accepted Journal Date: **2022.08.25**

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(56) Related Art
WO 2012/101106 A1
WO 2016/131133 A1
WO 2015/039225 A1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 July 2020 (23.07.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/149749 A1

(51) International Patent Classification:
A01N 65/00 (2009.01) *A01N 25/02* (2006.01)
A01N 65/40 (2009.01) *A01P 1/00* (2006.01)
A01N 59/00 (2006.01)

(21) International Application Number:
PCT/NZ2019/050124

(22) International Filing Date:
16 September 2019 (16.09.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

749953	14 January 2019 (14.01.2019)	NZ
755033	01 July 2019 (01.07.2019)	NZ

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

WO 2020/149749 A1

(54) Title: TREATMENT OF PLANTS AGAINST DISEASE.

(57) Abstract: Averting plant diseases is an ongoing battle in the agricultural and horticultural industries. Some diseases are minor; however others such as Xanthomonas present a serious problem, causing significant adverse economic impact. It is an object of the invention to go at least some way towards addressing this or to provide the public with a useful choice. The invention comprises a method of treating a plant against disease resulting from pathogens of Proteobacteria (eg Xanthomonas), comprising applying to the plant fatty acid and silicate.

TITLE

Treatment of plants against disease.

FIELD OF THE INVENTION

A preferred form of the invention relates to the treatment of plants against disease caused by pathogens of Proteobacteria (for example *Xanthomonas* bacteria).

A particularly preferred form of the invention relates to the treatment of cruciferous vegetables and their plant parts to prevent or reduce infection by 'black rot' caused by *Xanthomonas campestris pv. campestris.*

BACKGROUND

Averting plant diseases is an ongoing battle in the agricultural and horticultural industries. Some diseases are minor; however others such as *Xanthomonas* present a serious problem, causing significant adverse economic impact.

Xanthomonas species can cause cankers, bacterial spots and blights on leaves, stems, branches and fruits in a wide variety of plant species. Pathogenic species show high degrees of specificity and some are split into multiple pathovars, a species designation based on host specificity.

Diseases caused by *Xanthomonas* are of particular concern to growers of a wide range of crops including, but not limited to, cruciferous vegetables such as cabbage, broccoli, cauliflower etc., solanaceae vegetables such as tomatoes, peppers etc, tree crops such as citrus, stone fruits etc., nut crops such as walnut, hazelnut, etc, and grains such as wheat, barley or rice.

Spraying agricultural treatments is one of the more effective methods for managing infection by for the prevention or suppression of disease symptoms caused by *Xanthomonas* bacteria. They fall into three main groups depending on the plant to be treated and the circumstances of the infection.

Copper-based fungicides are commonly used to treat diseases caused by *Xanthomonas,* but in general they cannot be used long term as they may lead to undesirable levels of copper accumulating in the surrounding soil. Further, *Xanthomonas* bacteria can become too readily resistant to copper in certain crops,

therefore requiring higher rates to keep control of the disease. Copper can also be quite toxic to certain important soil organisms.

There is a relatively limited range of antibiotics available for treatment of plant diseases, and their long-term use heightens the risk of plants becoming resistant to them. Additionally there are often objections to these treatments based on the fear of humans acquiring resistance to the antibiotics, ie through consuming food produced using them. Antibiotics commonly used for treating diseases caused by *Xanthomonas* comprise *streptomycin* and *kasugamycin* based products.

There are some so-called 'soft' pesticide alternatives that require no withholding period because of their lack of any significant residual toxicity. Many are in the category of 'biologicals', which are organisms that prevent or influence the disease, or elicit heightened plant resistance to the disease. In many cases, when they are tested against either copper or antibiotic applications, many biologicals or 'elicitors' fall short in terms of efficacy. In some cases their mode of action requires particular climatic conditions, which may or may not exist in the environment at hand.

OBJECT OF THE INVENTION

It is an object of preferred embodiments of the invention to at least go some way towards averting plant diseases caused by pathogens of Proteobacteria, for example *Xanthomonas* bacteria, and particularly *Xanthomonas campestris pv. camprestris.* While this object applies to preferred embodiments, it should not be seen as a limitation on any claims expressed more broadly. The object of the invention *per se* is simply to provide the public with a useful choice.

DEFINITIONS

The term "comprising" or derivatives thereof, eg "comprises", if and when used in this document in relation to a combination of features should not be seen as excluding the option of additional unspecified features or steps. In other words, the term should not be interpreted in a limiting way.

SUMMARY OF THE INVENTION

According to one aspect of the invention there is provided a method of treating a plant against disease resulting from pathogens of Proteobacteria, for example from *Xanthomonas*, comprising applying to the plant:

- fatty acid in soap form; and
- silicate;

wherein the fatty acid is in solution or in suspension in water.

Optionally the method comprises applying a composition comprising the fatty acid and the silicate.

Optionally the *Xanthomonas is bacterial and comprises Xanthomonas campestris pv. campestris.*

The fatty acid and silicate kill, inhibit, directly control or eliminate the *Xanthomonas* bacteria.

Optionally the fatty acid comprise one or more of:

- sodium salt; and
- potassium salt.

Optionally the fatty acid comprises fat of animal origin.

Optionally the fatty acid comprises oil of plant origin.

Optionally the fatty acid comprises fat and oil of plant or animal origin.

Optionally fatty acid comprises one or more of the following-

- Caproic Acid
- Caprylic Acid
- Capric Acid
- Lauric Acid
- Myristic Acid
- Palmitic Acid
- Stearic Acid
- Oleic Acid

- Linoleic Acid
- Linolenic Acid
- Arachidic Acid

Optionally fatty acid comprises one or more of the following*-

- C6:0 : Caproic Acid
- C8:0 : Caprylic Acid
- C10:0 : Capric Acid
- C12:0 : Lauric Acid
- C14:0 : Myristic Acid
- C16:0 : Palmitic Acid
- C18:0 : Stearic Acid
- C18:1 : Oleic Acid
- C18:2 : Linoleic Acid
- C18:3 : Linolenic Acid
- C20:0 : Arachidic Acid

* The number immediately following the "C" term notes the number of carbon atoms in the molecule, and the number immediately after that designates the number of double bonds in the carbon chain. So for example "C6:0 Caproic acid" indicates that the molecule has '6' carbon atoms and '0' double bonds.

Optionally the silicate is water soluble.

Optionally the silicate is in the form of metallic salt.

Optionally the silicate comprises one or more of:

- potassium silicate;
- sodium silicate; and
- lithium silicate;

Optionally the molar ratio of the silicate ranges from 2.0 to 3.3. By way of example, if the silicate is potassium silicate and the molar ratio is 2.0, this means it contains 2.0 mol of SiO_2 for every 1 mol of K_2O. And if the silicate is potassium silicate at a molar ratio of 3.3, it contains 3.3 mol of SiO_2 for every 1 mol of K_2O.

Optionally the plant comprises one or more of a fruit, vegetable, nut, flower, grain or tree.

Optionally the fruit comprises one or more of citrus, peaches, nectarines, apricots, plums, cherries, tamarillos, pomegranates and berry fruit.

Optionally the vegetable comprises one or more of lettuce, brassicas, cucurbits, tomato, capsicum, chili, potato, sweet potato, carrots, beet, spring onions, leeks, beans and peas.

Optionally the grain comprises one or more of wheat, maize, sorghum, oats, rice and barley.

Optionally the tree comprises an ornamental variety selected from one or more of begonia, roses, ivy, geranium and poinsettia.

According to a further aspect, the invention comprises the use of:

- fatty acid; and
- silicate;

in the preparation of a composition for treating a plant against disease resulting from pathogens of Proteobacteria, for example from *Xanthomonas (eg Xanthomonas campestris pv. Campestris).* Preferably the fatty acid and/or silicate and/or plant are as per any of the options set out above.

DRAWINGS

Some preferred embodiments of the invention will now be described by way of example and with reference to the accompanying drawings, of which:

Figures 1-7 graph, logarithmically, the bacterial count in the presence of solutions of potassium soap alone, potassium silicate alone and a number of concentrations of individual potassium soaps and potassium silicate, and the efficacy effect achieved by the various concentrations of potassium silicate to various concentrations of potassium soap when used against *Xanthomonas campestris pv. campestris*.

DETAILED DESCRIPTION

In a preferred embodiment of the invention there is a composition for treating plants as above, against diseases as above. The composition is in the form of a solution for spraying, consisting of components as listed in the following examples.

Example 1

Component	Amount	Function
18.2 % w/v fatty acid potassium salt in water (ie 182 g potassium salt of fatty acid per litre of water)	0.08 - 2L	Active ingredient
A 44% w/v potassium silicate water solution (ie 440 g potassium silicate per litre of water)	21.6 – 540 mL (approximately 80ppm to 2000ppm Silica)	Active ingredient
Water	100L	Diluent - solvent

To produce the above composition, the silicate solution is added to about ¾ of the total water with stirring. The fatty acid potassium salt (in salt form) is then added with stirring. The balance of the water is then added with stirring.

The composition is in the form of a spray mixture ready to apply to plants by way of a manual or machine sprayer. Spraying is preferably liberal, such that excess composition runs off substantially all plant surfaces at critical plant growth stages, before disease occurs.

Example 2

The table below lists a number of specific prototype soap formulations produced in accordance with preferred embodiments of the invention.

Formulation	Contents
NS1	Potassium soap derived from fully refined, bleached and deodorised coconut oil (RBD Coconut Oil from Oilseed Products NZ Ltd).
NS2	Potassium soap derived from fully refined, bleached deodorised palm olein (RBD Palm Olein from Oilseed Products NZ Ltd).

The formulations NS1 and NS2 were produced by saponification. In this regard 1.63 kg of the oil component in each case was reacted with 420g of potassium hydroxide in 2.5L water. To assist the reaction, 360g of liquid potassium soap was added to the oil prior to the addition of the potassium hydroxide. The resulting concentrated solution was then buffered to a pH of approximately 10 using citric acid based buffer. Approximately 5 L of water was then added to make each formulation up to a final volume of 10 L. The amount of potassium salt of fatty acid in each of the "NS" soap formulation came out at approximately 18% w/v, or in other words 180 g/L soap per litre of water.

The fatty acid profile for NS1 and NS2 are generally as follows:

NS1	**Proportion % w/w**
• C6:0 : Caproic Acid	0-1.0
• C8:0 : Caprylic Acid	8.0
• C10:0 : Capric Acid	6.0
• C12:0 : Lauric Acid	47.0
• C14:0 : Myristic Acid	18.0

• C16:0 : Palmitic Acid	9.0
• C18:0 : Stearic Acid	3.0
• C18:1 : Oleic Acid	6.0
• C18:2 : Linoleic Acid	2.0

NS2	**Proportion %w/w**
• C12:0 : Lauric Acid	• 0-1.0
• C14:0 : Myristic Acid	• 0.5-1.5
• C16:0 : Palmitic Acid	• 37.0-42.0
• C18:0 : Stearic Acid	• 3.0-5.5
• C18:1 : Oleic Acid	• 40.0-45.0
• C18:2 : Linoleic Acid	• 9.0-13.0
• C18:3 : Linolenic Acid	• 0.0-1.0
• C20:0 : Arachidic Acid	• 0.0-1.0

These NS1 and NS2 prototype soap formulations were used in a number of *in-vitro* studies, both individually and in combination with potassium silicate, as described below.

In Vitro Treatment of *Xanthomonas campestris pv. campestris*

Laboratory trials were run to compare the effectiveness of certain embodiments of the invention against *Xanthomonas campestris pv. campestris*. The trial measured the bacterial count observed in the presence of the following test compositions:

- NS1 and NS2 alone (each approximately 18% w/v potassium salts of fatty acids);
- potassium silicate alone (concentration 44% w/v, molar ratio 2.2);
- the combination of each 'NS..' component and silicate;
- Kasugamycin (an industry standard antibiotic);

- Streptomycin (an industry standard antibiotic)
- Distilled water alone.

The test compositions are listed in the table below.

Product	Units	Treatment Rates																Total
NS1	L/100 L	0.08	0.4	1	2													4
NS2	L/100 L	0.08	0.4	1	2													4
Potassium silicate	mL/100 L	21.6	108	270	540													4
NS1 + Potassium silicate	L/100 L NS1	0.08	0.08	0.08	0.08	0.4	0.4	0.4	0.4	1	1	1	1	2	2	2	2	16
	mL/100 L Potassium silicate	21.6	108	270	540	21.6	108	270	540	21.6	108	270	540	21.6	108	270	540	
NS2 + Potassium silicate	L/100 L NS2	0.08	0.08	0.08	0.08	0.4	0.4	0.4	0.4	1	1	1	1	2	2	2	2	16
	mL/100 L Potassium silicate	21.6	108	270	540	21.6	108	270	540	21.6	108	270	540	21.6	108	270	540	
Water		1																2
Streptomycin sulphate	g/100L	10.2																1
Kasumin (20 g/L kasugamycin)	L/100L	0.5																1
Total Treatment Number																		48

NS1, NS2 and the potassium silicate product were evaluated for their efficacy on an agar plate, in terms of their ability to control growth of Xanthomonas campestris pv. campestris at predetermined concentrations and combinations, a total of 53 treatments per replicate (see table above).

Two positive controls (Streptomycin and Kasumin (20g/L kasugamycin)) and a negative control (distilled water) were also included.

Each of the products was prepared to four times the required concentration. A 0.25 mL aliquot of each of these solutions was combined with 0.25 mL of potassium silicate (or water) and 0.5 mL of bacterial suspension making a total volume of 1 mL. The solutions containing the products and bacteria were then incubated for 1 h at 20°C prior to diluting in a ten-fold series in sterile distilled water down to 10^{-7}. The diluted solutions were then plated to Casitone-yeast extract agar (CYE agar) (Araújo et al. 2012), and incubated at 20°C until individual bacterial colonies could be enumerated.

Each solution was separately made as three true replicates. Treatment means for each replicate were averaged over the three replicates. Treatments were randomized but not statistically analysed as the large number of zeros nullified the ANOVA model. All data were presented on a logarithmic scale to enable differences in bacterial concentrations to be visualized.

The bacterial colony count results for each sample are as shown at **Figures 1-5**. While some preferred embodiments of the invention have been exemplified, it should be appreciated that modifications and improvements can occur without departing from the scope of the following claims.

In terms of disclosure, this document hereby discloses each item, feature or step mentioned herein in combination with one or more of any of the other item, feature or step disclosed herein, in each case regardless of whether such combination is claimed.

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CLAIMS

1. A method of treating a plant against disease resulting from *Xanthomonas* bacteria, comprising applying to the plant:
   - fatty acid in the form of soap; and
   - silicate;

   wherein the fatty acid is in solution or in suspension in water.

2. A method according to claim 1, comprising applying to the plant a composition comprising the fatty acid and silicate.

3. A method according to claim 1 or 2, wherein the disease results from *Xanthomonas campestris pv. Campestris*.

4. A method according to claim 1, 2 or 3, wherein the fatty acid and silicate kill the *Xanthomonas* bacteria.

5. A method according to claim 1, 2 or 3, wherein the fatty acid and silicate inhibit the *Xanthomonas* bacteria.

6. A method according to claim 1, 2 or 3, wherein the fatty acid and silicate directly control the *Xanthomonas* bacteria.

7. A method according to claim 1, 2 or 3, wherein the fatty acid and silicate directly eliminate the *Xanthomonas* bacteria.

8. A method according to any one of the preceding claims, wherein the fatty acid is in the form of one or more of:
   - sodium salt; and
   - potassium salt.

9. A method according to any one of the preceding claims, wherein the fatty acid comprises fat of animal origin.

10. A method according to any one of the preceding claims, wherein the fatty acid comprises oil of plant origin.

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11. A method according to any one of the preceding claims, wherein fatty acid comprises fats and oil of plant or animal origin.

12. A method according to any one of the preceding claims, wherein the fatty acid comprises one or more of the following-

- Caproic Acid
- Caprylic Acid
- Capric Acid
- Lauric Acid
- Myristic Acid
- Palmitic Acid
- Stearic Acid
- Oleic Acid
- Linoleic Acid
- Linolenic Acid
- Arachidic Acid

13. A method according to any one of the preceding claims, wherein the fatty acid comprises one or more of the following-

- C6:0 : Caproic Acid
- C8:0 : Caprylic Acid
- C10:0 : Capric Acid
- C12:0 : Lauric Acid
- C14:0 : Myristic Acid
- C16:0 : Palmitic Acid
- C18:0 : Stearic Acid
- C18:1 : Oleic Acid
- C18:2 : Linoleic Acid
- C18:3 : Linolenic Acid
- C20:0 : Arachidic Acid

14. A method according to any one of the preceding claims, wherein the silicate is water soluble.

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15. A method according to any one of the preceding claims, wherein the silicate is in the form of metallic salt.

16. A method according to any one of the preceding claims, wherein the silicate comprises one or more of:

- potassium silicate;
- sodium silicate;
- lithium silicate;

17. A method according to any one of the preceding claims, wherein the molar ratio of the silicate is from 2.0 to 3.3.

18. A method according to any one of the preceding claims, wherein the plant comprises one or more of a fruit, vegetable, nut, flower, grain and tree.

19. A method according to claim 18, wherein the fruit comprises one or more of citrus, peaches, nectarines, apricots, plums, cherries, tamarillos, pomegranates and berry fruit.

20. A method according to claim 18, wherein the vegetable comprises one or more of lettuce, brassicas, cucurbits, tomato, capsicum, chili, potato, sweet potato, carrots, beet, spring onions, leeks, beans and peas.

21.A method according to claim 18, wherein the grain comprises one or more of wheat, maize, sorghum, oats, rice and barley.

22. A method according to claim 16, wherein the plant comprises an ornamental variety selected from one or more of begonia, roses, ivy, geranium and poinsettia.

23. The use of:

- fatty acid; and
- silicate;

in the preparation of a composition for treating a plant against disease resulting from *Xanthomonas* bacteria.

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24. A use as claimed in claim 21, wherein the disease comprises *Xanthomonas campestris pv. Campestris.*

25. The use according to claim 21 or 22, wherein the fatty acid is as described in any one or more of claims 5 to 12.

26. The use according to any one of claim 21, 22 or 23 wherein the silicate is as described in any one or more of claims 12 to 15.

Figure 1

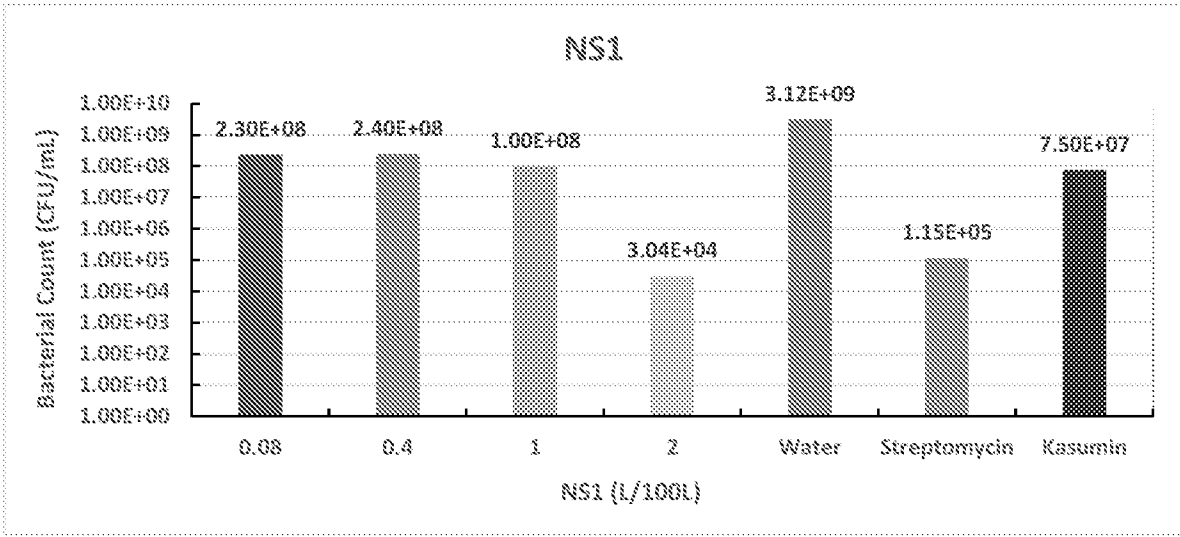


Figure 2

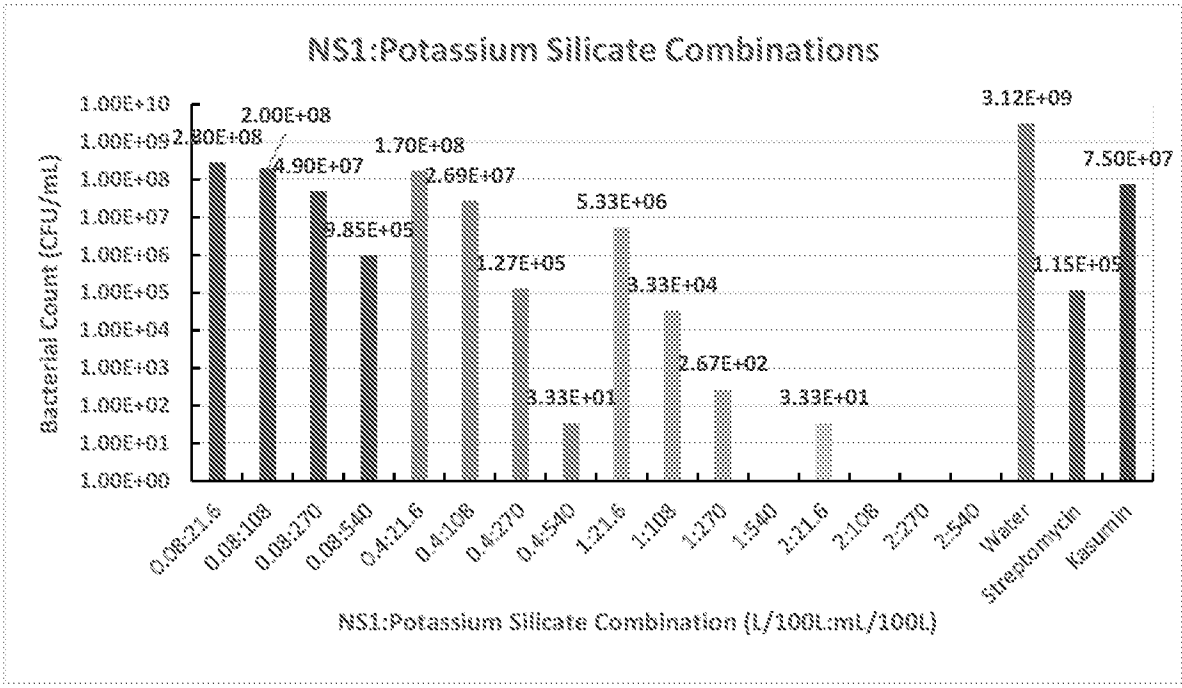


Figure 3

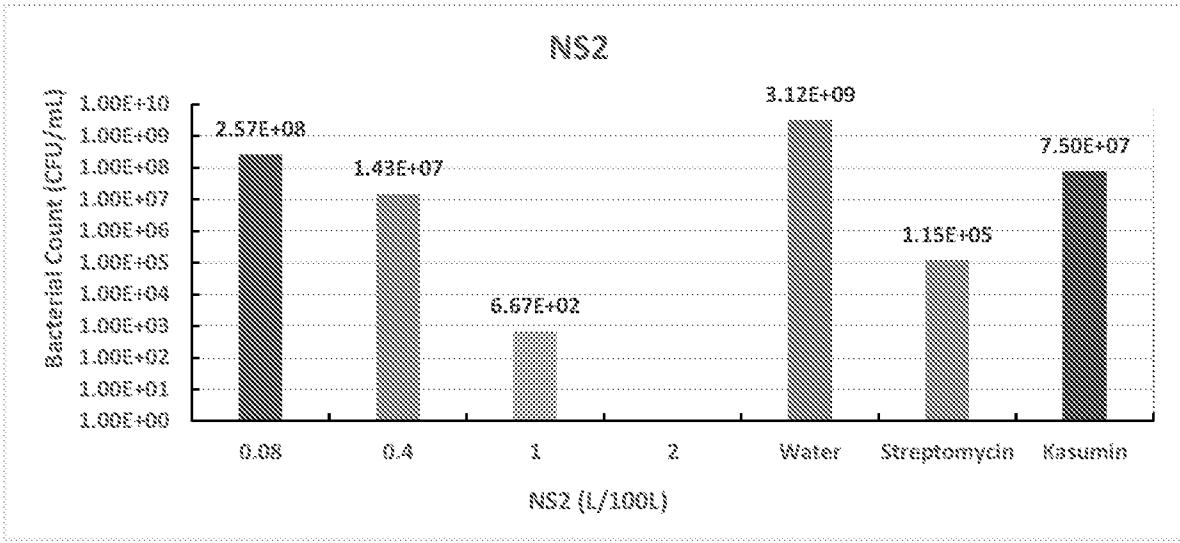


Figure 4

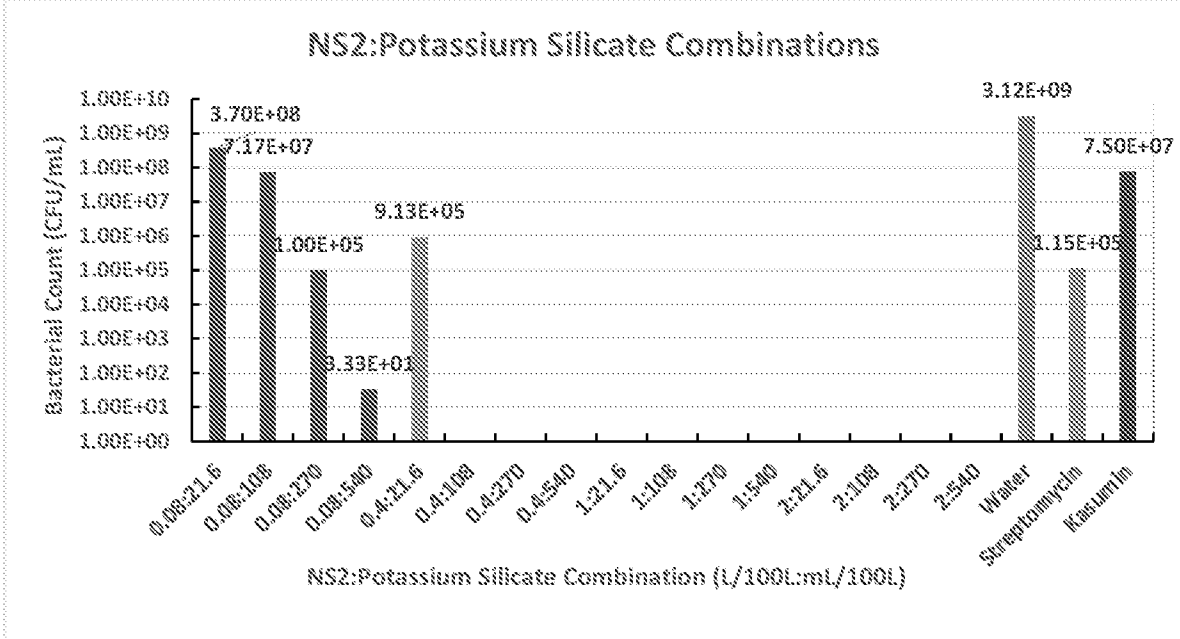


Figure 5

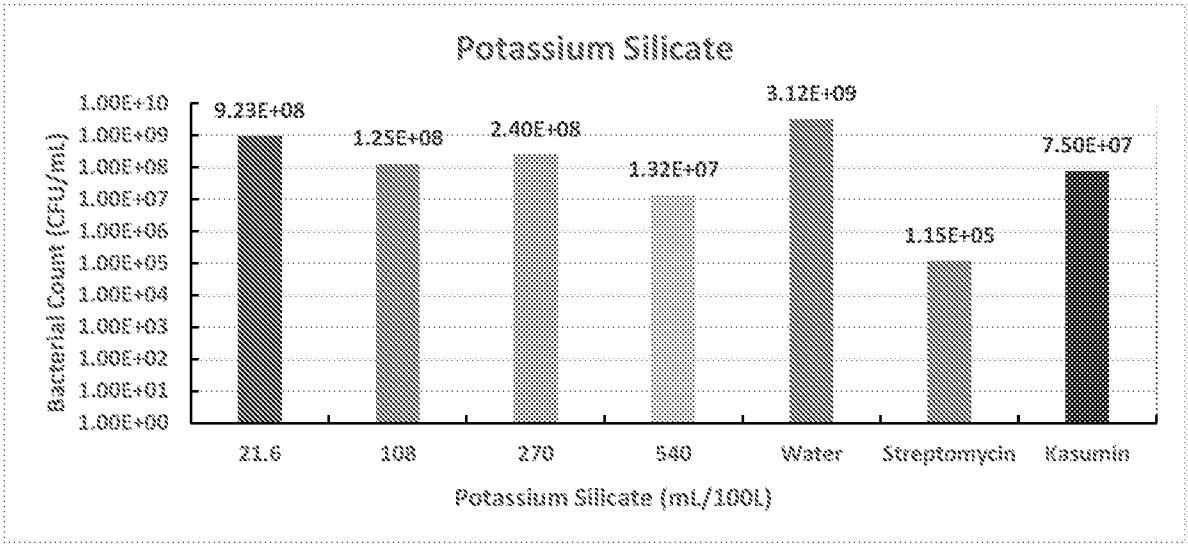


Figure 6

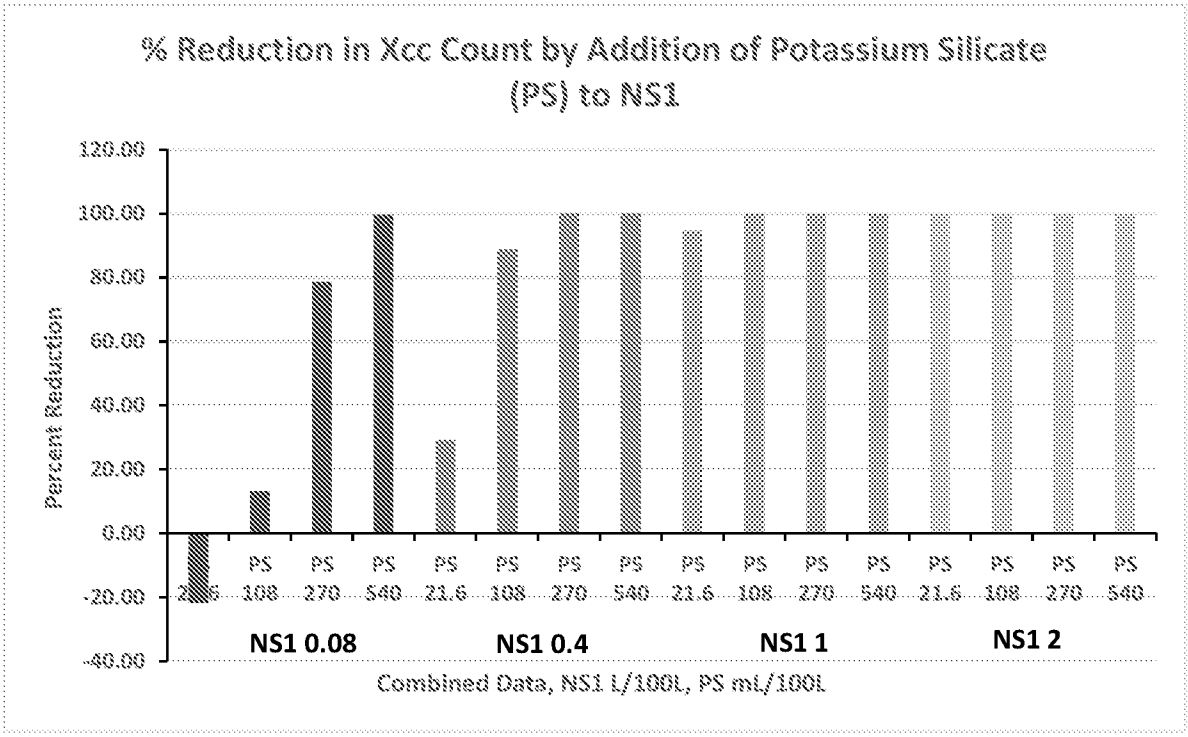
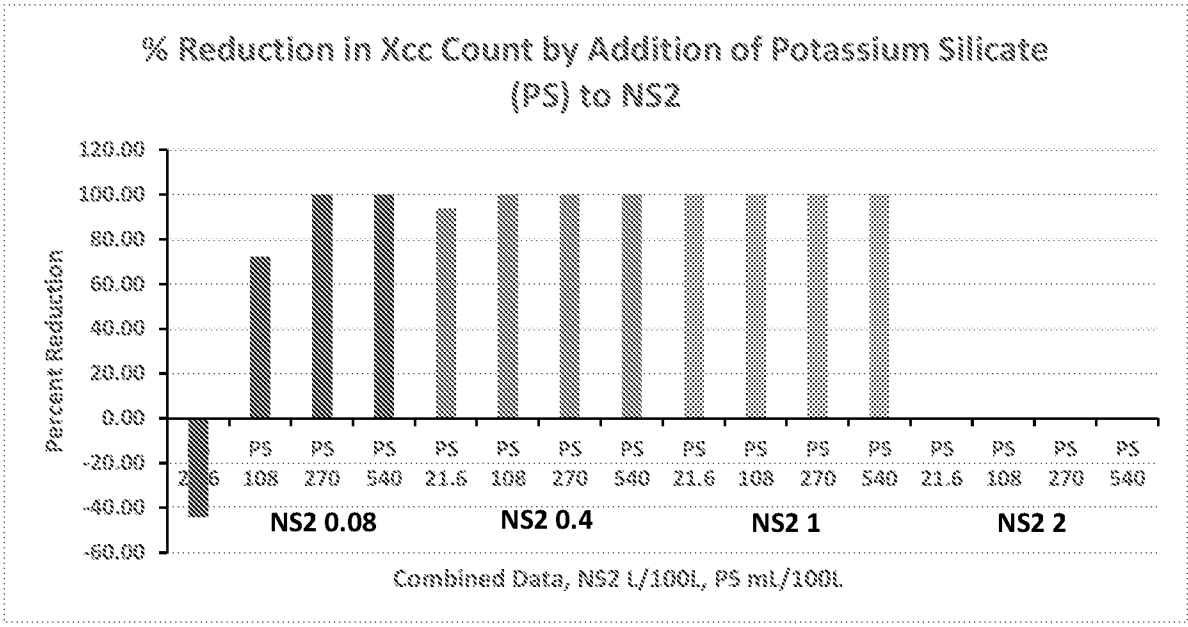


Figure 7


% Reduction in Xcc Count by Addition of Potassium Silicate (PS) to NS2
Percent Reduction
120.00
100.00
80.00
60.00
40.00
20.00
0.00
-20.00
-40.00
-60.00
PS 108
PS 270
PS 540
PS 21.6
NS2 0.08
NS2 0.4
NS2 1
NS2 2
Combined Data, NS2 L/100L, PS mL/100L