

Description

A METHOD AND COMPOSITION FOR TREATING BEES AGAINST VARROA MITES FIELD OF THE INVENTION

This invention relates to a method and composition for the treatment of bees against parasitic infestation, particularly, but not exclusively to treatment of honey bees against infestation by Varroa mites including Varroa destructor and Varroa Jacobsoni.

BACKGROUND TO THE INVENTION Honey bees {Apis spp.} are an important component of the agricultural industry, directly providing honey for the food markets as well as playing a key role in pollination, which in turn is essential in the functioning of ecosystems and optimisation of agricultural yields. It is estimated that 60 - 80% percent of horticultural and agricultural crops requires honey bees for pollination as they are either self-incompatible or only achieve commercially adequate yield through pollination vectors.

Recent trends indicate that there is an increase in mortality of the honey bee, the problem being so severe that the White House in the United States has declared a state of emergency following the negative effects on the industry caused predominantly by the Varroa mite. It has also been observed in China that human intervention has been deemed necessary and pollination by hand has been introduced owing to the drastic demise of bee populations in certain areas. The increased mortality has been linked to, amongst other, infestation of bees by ectoparasites such as mites, more particularly the parasitic mite Varroa destructor { Varroa mite}. The increased bee mortality has also been linked to the effect of chemical mite treatments and other pesticides.

The Varroa mite reproduces in the brood cells of developing honey bees and feed on larvae and the haemolymph of developing and adult bees, resulting in compromised immunity and the transmission of secondary diseases such as deformed wing virus to larva and/or pupating bees. These result in death or severe deformity of the pupae and bees. The condition of a honey bee colony infested with Varroa mite is referred to as varroaosis.

Varroa mites have accordingly been implicated in colony collapse disorder. Current treatments for controlling Varroa mite infestation in honey bees include chemical treatments, topical treatments, natural product treatments and dietary supplements.

Chemical treatments include Apivar and Tua-fluvalinate. Topical treatments include the administration of confectionary/icing sugar, oxalic acid and other organic acids, 65% formic acid and Mite Away Quick Strips.

Natural product treatments and dietary supplementation include hops treatment, Vitamin C and ionic metals administration in which all of these are administered separately to the bees. Oral dosing of micro- and macro nutrients such as water/fat soluble vitamins and minerals such iron and magnesium have also been proposed.

Alternative methods for control of bee mites, particularly Varroa mites, have also been developed. US2015/0133532 & WO2015/001336 describe the use of nucleic acid for the prevention and treatment of bee mite infestation ; and US2009/01 18214 describes the uses of nucleic acid for prevention and treatment of viral infections in honeybees. In addition, US2014/135281 describes the use of cryomazine (a triazine insect growth regulator used as an insecticide and an acaricide) and US2013/0338222 describes the use of spirodiclofen (a broad spectrum acaricide) for control of bee mites and for the reduction of bee mite infestations.

The disadvantages of current methods and compositions for treatment include lack of efficacy, generation of resistance by the Varroa mites, handling difficulties and contamination of beeswax. The methods and compositions are furthermore relatively expensive.

Miticides have also been known to have a negative effect on the honey bee's immune system which allows secondary infections to take place and in some instances these miticides kill the bees themselves.

Some treatments use genetically modified DNA constructs which may lead to the bees themselves and/or honey and other products derived from the affected bees falling into the category of genetically modified organisms (GMO's). The honey produced in such hives would in these circumstances be barred from export to regions that have imposed restrictions on the import of GMO's.

OBJECT OF THE INVENTION

It is accordingly an object of the present invention to provide a method and composition for the treatment of bees with which the above disadvantages will at least partially be overcome or alleviated and/or to provide a more useful alternative to the known methods and compositions.

SUMMARY OF THE INVENTION

According to a first aspect of the invention, there is provided a composition for the treatment of bees against parasitic infestation comprising a mixture of at least one Fenton's reagent to operatively produce free radicals, and a bee attractant in a suitable solution; the Fenton's reagent and/or the concentration thereof being selected such as to be metabolically tailored to be detrimental to the metabolism of the parasite. The composition is further selected such as to be complementary to the bee's metabolism, but detrimental to the parasite's metabolism.

The metabolism of the parasite is more particularly, the antioxidant defence metabolism. The bee attractant may be provided as a monosaccharide and/or disaccharide base including any one or more selected from the group consisting of glucose, sucrose, fructose, dextrose, honey and syrup. The invention further provides for the free radical producing Fenton's reagent, to comprise a transition metal and an organic compound, to drive reduction-oxidation (redox) reactions to generate free radicals, in particular hydroxide and oxygen radicals, using the organic compound which operatively acts as a free radical generator.

The transition metal, such as iron or copper, can be either in the ionic form or in a colloidal suspension. The transition metal, when in colloidal suspension, may be coated with a polymer such as polyethylene glycol (PEG), polyvinylpyrrolidone (PVP) and polyethyleneimine (PEI), including block-co-polymer and dendrimer variants thereof, to operatively regulate the rate of release of the free radicals using the Fenton's reagent.

The organic compounds which can drive the Fenton's reaction as a reagent, may include any one or more of the group consisting of ascorbic acid (or any salt or derivative thereof), quercetin, epigallo-catechin-3-gallate, catechins, gallic acid, resveratrol and other non-toxic aromatic compounds used for inducing the Fenton's reaction; and which are provided at a pH and concentration which favours a pro-oxidative action, preferably between 3 and 11. The invention yet further provides for the composition to comprise: - from 20-70% (w/v) sugar solution, such as monosaccharides, disaccharides and dextroses which proves attractive for the bee;

- from 0.01 -20.0% (v/v) organic acid (or any salt or derivative thereof); and

- transition metal colloids coated with biocompatible molecules in a concentration range of 0.1 mg/mL to 50mg/mL; and

- may optionally include ionic iron (ferric and ferrous) and/or ionic copper (cuprous and cupric), in ranges from 0.01 - 5mg/mL.

The invention further provides for the parasitic infestation to be a mite infestation, in particular a Varroa destructor and/or Varroa Jacobsoni infestation.

According to a second aspect of the invention there is provided a method for the treatment of bees against parasitic infestation including the step of making available to bees in need thereof an effective amount of the composition according to the first aspect of the invention in a form and concentration such as to accumulate free radicals in the parasite, so as to be detrimental to the metabolism, more particularly, the antioxidant defence metabolism of the parasite.

The composition is further selected such as to be complementary to the bee's metabolism, but detrimental to the parasite's metabolism.

The metabolism of the parasite is more particularly, the antioxidant defence metabolism. The invention yet further provides for the composition to be administered in a two part form wherein the first part comprises colloidal transition metal component (and/or the ionic iron/copper) part of the Fenton's reagent and bee attractant in suitable solution; and wherein the second part comprises the organic compound part of the Fenton's reagent and bee attractant in suitable solution.

The invention yet further provides for the first part to be administered to the bees, followed by the second part to be administered in selected time periods thereafter, preferably at intervals of 1, 3, 6, 12 and 24 hours.

The invention further provides for the composition to be administered to the bees in a non-honey production period.

The invention further provides for the step of making available to bees in need thereof an effective amount of the composition to include a preceding step of administering to the bees a metabolic stressor to operatively enhance the selective production of free radicals such as to sensitize the parasite to the composition.

The preceding step is preferably taken 12 to 24 hours before the subsequent step.

The metabolic stressor may include the acetaminophen class of compounds and other related compounds known to deplete endogenous antioxidants such as glutathione, reduced (GSH) or oxidised (GSSG). The parasitic infestation may further be in the form of a mite infestation, such as any one or more of a Varroa destructor and Varroa Jacobsoni infestation.

According to a third aspect of the invention there is provided a kit for the treatment of bees against parasitic infestation comprising a composition according to the first aspect of the invention; and a feeding tray having a plurality of dispensers each for making available to bees in need thereof an effective amount of the said composition. The kit may further include a receptacle at the underside of the tray to collect fallen mites.

The invention further provides for the kit to include a metabolic stressor to operatively enhance the production of free radicals such as to sensitize the parasite to the composition.

The metabolic stressor may include the acetaminophen class of compounds and other related compounds known to deplete endogenous antioxidants such as glutathione, reduced (GSH) or oxidised (GSSG).

The invention further includes the use of the composition according to the first aspect of the invention, and the kit according to the third aspect of the invention in a method for the treatment of bees against parasitic infestation. BRIEF DESCRIPTION OF THE PHOTOGRAPHS AND DRAWINGS The invention will now be described further, by way of example only, with reference to accompanying photographs and drawings wherein: figure 1 depicts photographs of larvae and honeybees carrying Varroa destructor mites; figure 2 is a diagrammatic representation of the reaction scheme for producing free radicals according to the invention using colloidal iron oxide and free ascorbic acid as an example model; figure 3 illustrates a feeding tray for use in administering the composition of the invention to bees; figures 4 & 5 depict diagrams showing the number and percentage of Varroa fall from the bees treated with a composition in accordance with a first preferred embodiment of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides a composition and method for the treatment of bees against parasitic infestation, more particularly infestation by mites such as Varroa destructor and Varroa Jacobsoni. As used in the specific description of the invention, the term "bee" refers to both an adult and pupal cell of the honey bee (*Apis* spp.). In addition, the specific embodiment describes the parasite as Varroa mites, but the term parasite may include obligate and facultative parasites, endo- and ectoparasites. Parasitic infestation will therefore include infestation of any of the aforementioned parasites.

As shown in figure 1, the Varroa destructor ("Varroa mites") 10 attach to the larvae 12 and bees 14. According to a first embodiment of the invention, the composition comprises Fenton's reagent(s) to operatively produce free radicals in a bee attractant solution suitable for administering to a bee. The free radical producing reagent includes any compound which drives the Fenton reaction to generate free radicals. The free radical producing Fenton reagent(s), comprising a transition metal and an organic compound, drives reduction-oxidation (redox) reactions to generate free radicals, in particular hydroxide and oxygen radicals, using an organic compound which operatively acts as a free radical generator.

The transition metal, e.g. iron or copper, can be either in the ionic form or in a colloidal suspension. The organic compounds which drive the Fenton reaction as a reagent, may include quercetin, epigallo-catechin-3-gallate, catechins, gallic acid, resveratrol and any other reported non-toxic aromatic compounds used for inducing the Fenton reaction. These components are provided in a suitable solution together with a bee attractant in the form of a monosaccharide and/or disaccharide (eg. glucose, sucrose, fructose, dextrose, honey or syrup) which is administered to the bees. The composition operatively provides a mixture of hydrogen peroxide (H_2O_2) as an oxidation agent and a transition-metal ion as a catalyst, more particularly iron (Fe^{2+}). The reaction operatively forms reactive free radicals such as hydroxyl ($HO\cdot$) as well as a higher oxidation state of the transition-metal ion (Fe^{3+}). The hydroxyl radical has an unpaired electron making it highly reactive, relatively nonspecific oxidant. This reaction is illustrated in Figure 2. Ionic copper (Cu^{2+} and/or Cu^+) is also included in the composition to enhance the generation of free radicals thereby forming an operative enhancer. The composition also includes a biodegradable colloid such as iron oxide (Fe_3O_4). The colloid is coated with a polymer such as polyethylene glycol (PEG), polyvinylpyrrolidone (PVP) and polyethyleneimine (PEI) to operatively regulate the rate of release of the free radicals. The surface coating can be neat molecules or combinations in various covalent attachments such as block-co-polymers and dendrimer variants of the above mentioned polymers.

In a preferred embodiment of the invention, the composition comprises (provided as a 50ml_ formulation):

- from 20-70% (%m/v) sugar solution, such as a monosaccharide, disaccharide, dextroses solution which proves attractive for the bee;
 - from 0.01 -20.0% (%v/v) organic acid (or any salt or derivative thereof) or derivatives thereof; and
 - transition metal colloids coated with biocompatible molecules in a concentration range of 0.1/mL to 50mg/mL.
- Optionally, the composition may include ionic iron (ferric and ferrous) and/or ionic copper (cuprous and cupric), in ranges from 0.01 -5mg/mL.

The composition is provided at a pH range of between 3 and 11 to favour the pro-oxidative actions of the composition. The iron colloids are stored in a 2.5-5mM ascorbic acid (pH 7) solution to prevent oxidation of the Fe^{2+} on the surface of the colloids. Prior to use the colloids are magnetically washed and suspended in distilled water.

The aforementioned compounds are mixed into a solution to form a colloidal solution which is subsequently aliquoted into perforated 50ml_ polypropylene tubes 16 for administering to the bees.

As shown in figure 3, a feeding tray 18 including the tubes 16 is provided to the bees (not shown). The bees enter through suitable openings 20 and feed on the composition. The composition may be administered to the bees in any alternative and known manner. In particular, administration to bees on a larger scale will in some instances be required and in such cases will be provided in a suitable alternative manner. Upon feeding of the composition to the bees, the composition distributes through the haemolymph of the bee. Here, the Fenton reaction may occur, leading to hydroxyl radical which acts as an anti-oxidant in the bee leading to the known advantages to the bee.

As the Varroa mites parasitize on the bee the composition, including the hydroxyl radicals, also accumulates in the Varroa mites. The accumulation of the free radicals in the Varroa mite is based on work done by Giese and Giese (2014). Giese et al (2014) demonstrated that a genetic construct, tailored to induce green fluorescent protein expression in the bee upon oral ingestion in a 65% sucrose carrier solution of said construct, showed green fluorescence in the bee 12-16h post ingestion. Over a course of 24h the green fluorescence in the bee disappeared, where green fluorescence was observed in the Varroa mite. Demonstrating the Varroa mite was parasitically ingesting the bee haemolymph, accumulating the green fluorescent protein in the varroa mite. In the invention however it was surprisingly found that the free radicals are more pronounced in the Varroa mites. The bee accordingly acts as a hemolymph filter and the free radicals accumulate in the mites but not necessarily in the bee. The free radicals in the mites act as a pro-oxidant causing the mites to either disengage and fall from the bee or even die.

The floor 22 of the feeding tray 18 is provided with suitably sized openings to a receptacle 24 at the underside of the tray 18 to collect the fallen mites. The receptacle 24 is removed to dispose of the mites. The composition is administered as a single treatment wherein all the components described are pre-mixed. Alternatively, the composition is administered in a two part form wherein the first part comprises the colloidal transition metal component (and/or the ionic iron/copper) part of the Fenton's reagent and bee attractant in suitable solution; and the second part comprises the organic compound part of the Fenton's reagent and bee attractant in suitable solution. In this instance the first part will be administered to the bees, followed by the second part to be administered in selected time periods thereafter, preferably at intervals of 1, 3, 6, 12 and 24 hours. This facilitates the release and accumulation of free radicals in the mites in a controlled manner.

The composition is further coated with any other suitable polymer in order to control the rate of free radical production according to specific requirements. As mentioned, the colloid variant is coated with a polymer such as polyethylene glycol (PEG), polyvinylpyrrolidone (PVP) and polyethyleneimine (PEI), including block-co-polymer and dendrimer variants of the above mentioned polymers, to operatively regulate the rate of release of the free radicals using the Fenton's reagent.

In use, the composition is administered to the bees on a weekly or bi-weekly basis and preferably in a non-honey production season such as during winter.

As shown in figures 4 and 5, two formulations of the composition were prepared and administered to a number of bees. These formulations were reasonably effective, as measured against the controls, in effecting the fall of Varroa mites from the bees over a period of 72 hours. In particular, Formulation 2 provided a 90% success rate. The formulation in this embodiment, was 8mg/mL_ vitamin C-capped iron colloids for S1-S4. The formulations differed with vitamin C added as follows: S1 : 0.5%, S2: 1 %, S3: 1.5%, S4: 2%.

The invention also makes provision for the enhancement of the Fenton treatment by using a metabolic stressor on the Varroa mite to enhance the selective production of free radicals, thereby sensitizing the mite to the effects of the

Fenton treatment. The metabolic stressor can be selected from the acetaminophen (paracetamol) class of compounds and other related compounds known to deplete endogenous antioxidants such as glutathione, reduced (GSH) or oxidised (GSSG).

The metabolic stressor will be administered 12 to 24 hours before the composition according to the invention is administered to the bees, using a similar bee attractant formulation as described herein or any other suitable bee attractant.

The invention therefore also provides a suitable kit comprising the feeding tray and composition as described for the treatment of bees against parasitic infestation for example a Varroa mite infestation.

The invention uses the bee's inherent biochemistry and metabolism against parasites such as Varroa mites and accordingly has limited side effects on the bee. The formulation of the composition will be provided by scientific and rational design wherein the free radicals preferentially cause damage in the Varroa mite with limited damage in the bee, by exploring the differences in the role of the free radical in the bee and Varroa mite metabolism, more particularly to the antioxidant defence metabolism. The form and/or concentration of the composition may accordingly be adapted as such without departing from the scope of the invention. It is also envisaged that the invention may be extended to bacterial, viral and fungal infection of bees.

The invention provides the use of a bio-degradable, biocompatible nanomaterial in the treatment of the bees without any genetic modification of the bees or any long term bio-accumulation of products in the neither bees nor transfer thereof into honey.

The composition is furthermore relatively much more affordable as compared to the prior art.

The composition according to the invention thus comprises compounds which operatively enhances and/or modulates the Fenton reaction to produce free radicals. In other words, the invention relates to providing/creating Fenton's reagents in the composition for bees, which induces the Fenton cycle which result in the aforementioned advantages. It will accordingly be understood that any composition or combination of compositions which will drive the Fenton reaction/ produce free radicals in a solution suitable for administering to bees may be provided without departing from the scope of the invention. The same will apply to the method of the invention in that any method of enhancing/modulating the Fenton reaction to produce free radicals in the bee will be within the scope of the current invention.

Claims

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A composition for the treatment of bees against parasitic infestation comprising a mixture of at least one Fenton's reagent to operatively produce free radicals, and a bee attractant in a suitable solution; the Fenton's reagent and/or the concentration thereof being selected to be detrimental to the metabolism of the parasite.

The composition according to claim 1, wherein the composition is selected such as to be complementary to the bee's metabolism, but detrimental to the parasite's metabolism.

The composition according to claims 1 or 2, wherein the metabolism of the bee is the antioxidant defence metabolism.

The composition according to claim 1, 2 or 3, wherein the bee attractant is provided as any one or more of a monosaccharide and disaccharide base including any one or more selected from the group consisting of glucose, sucrose, fructose, dextrose, honey and syrup.

The composition according to any one of claims 1 to 4, wherein the free radical producing Fenton's reagent comprises a transition metal and an organic compound to drive reduction-oxidation reactions to generate free radicals, in particular hydroxide and oxygen radicals, using the organic compound which operatively acts as a free radical generator.

6. The composition according to claim 5, wherein the transition metal, such as iron or copper, is in the ionic form or in a colloidal suspension.

7. The composition according to claim 6, wherein the transition metal, when in colloidal suspension, is coated with a polymer such as polyethylene glycol (PEG), polyvinylpyrrolidone (PVP) and polyethyleneimine (PEI), includes block-co-polymer and dendrimer variants thereof, to operatively regulate the rate of release of the free radicals using the Fenton's reagent.

8. The composition according to any one of claims 5 to 7, wherein the organic compound includes any one or more of the group consisting of ascorbic acid or any salt derivative thereof, quercetin, epigallo-catechin-3-gallate, catechins, gallic acid, resveratrol and other non-toxic aromatic compounds used for inducing the Fenton reaction; and which are provided at a pH and concentration which favours a pro-oxidative action, preferably between 3 and 11.

9. The composition according to claim 8 comprising of:

20 - 70% (%m/v) sugar solution, such as monosaccharides, disaccharides and dextroses, which proves attractive for the bee;

0.01 - 20.0% (%v/v) organic acid or any salt derivative thereof; and

transition metal colloids coated with the polymer in a concentration range of 0.1 mg/mL to 50mg/mL.

10. The composition according to claim 9, wherein the composition further includes ionic iron, ferric and ferrous, and/or ionic copper, cuprous and cupric, in ranges from 0.01 -5mg/mL.

11. The composition according to any one of the preceding claims, wherein the parasitic infestation is a mite infestation such as any one or more of a Varroa destructor and Varroa Jacobsoni infestation.

12. A method for the treatment of bees against parasitic infestation including the step of making available to bees in need thereof an effective amount of the composition according to any one of claims 1 to 10 in a form and

concentration such as to operatively accumulate free radicals in the parasite, such as to be detrimental to the metabolism of the parasite.

13. The method according to claim 1 2, wherein the composition is in a form and concentration such as to be complementary to the bee's metabolism, but detrimental to the parasite's metabolism.

14. The method according to claim 12 or 13, wherein the metabolism of the bee is the antioxidant defence metabolism.

15. The method according to any one of claims 1 2 to 14, wherein the step of making available to bees in need thereof an effective amount of the composition comprises administering the composition to the bees in a two part form, wherein the first part comprises colloidal transition metal component and/or the ionic iron/copper part of the Fenton's reagent and bee attractant in suitable solution; and wherein the second part comprises the Fenton's reagent and bee attractant in suitable solution together with a suitable colloid.

16. The method according to claim 1 5 wherein the first part is to be administered to the bees, followed by the second part to be administered in selected time periods thereafter, preferably at intervals of 1 , 3, 6, 12 and 24 hours.

17. The method according to any one of claims 1 2 to 16, wherein the step of making available to bees in need thereof an effective amount of the composition is provided in a non-honey production period.

18. The method according to any one of claims 1 2 to 17, wherein the step of making available to bees in need thereof an effective amount of the composition includes a preceding step of administering to the bees a metabolic stressor to operatively enhance the production of free radicals such as to sensitize the parasite to the composition.

19. The method according to claim 1 8, wherein the metabolic stressor is selected from the acetaminophen class of compounds and other related compounds known to deplete endogenous antioxidants such as glutathione, reduced (GSH) or oxidised (GSSG).

20. The method according to claim 1 8 or 1 9, wherein the preceding step is taken 12 to 24 hours before the step of making available to bees in need thereof an effective amount of the composition.

21 . The method according to any one of claims 12 to 20, wherein the parasitic infestation is a mite infestation, such as any one or more of a Varroa destructor and Varroa Jacobsoni infestation.

22. A kit for the treatment of bees against a parasitic infestation comprising a composition according to any one of claims 1 to 10, and a feeding tray having a plurality of dispensers each for making available to bees in need thereof an effective amount of the said composition.

23. The kit according to claim 22, wherein a receptacle is included at the underside of the tray to collect fallen mites.

24. The kit according to claim 22 or 23, wherein the kit further includes a metabolic stressor to operatively enhance the production of free radicals such as to sensitize the parasite to the composition.

25. The kit according to claim 24, wherein metabolic stressor includes the acetaminophen class of compounds and other related compounds known to deplete endogenous antioxidants such as glutathione, reduced (GSH) or oxidised (GSSG).

26. Use of the composition according to any one of claims 1 to 10 in a method for the treatment of bees against parasitic infestation.

27. Use of a metabolic stressor to operatively enhance the production of free radicals such as to sensitize the parasite prior to use of the composition according to any one of claims 1 to 10 in a method for the treatment of bees against parasitic infestation.

28. Use of claim 27, wherein the metabolic stressor includes the acetaminophen class of compounds and other related compounds known to deplete endogenous antioxidants such as glutathione, reduced (GSH) or oxidised (GSSG).

29. Use of claim 26, 27 or 28, wherein the parasitic infestation is a mite infestation such as any one or more of a Varroa destructor and Varroa Jacobsoni infestation.

30. The composition according to claim 1 , substantially as herein described and exemplified and/or described with reference to the accompanying figures.

31 . The method according to claim 1 2, substantially as herein described and exemplified and/or described with reference to the accompanying figures.

32. The kit according to claim 22, substantially as herein described and exemplified and/or described with reference to the accompanying fig