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(54) Title: COMPOSITION FOR THE TREATMENT AND/OR PREVENTION OF THE BLACK SIGATOKA FUNGAL DISEASE IN BANANA CROPS

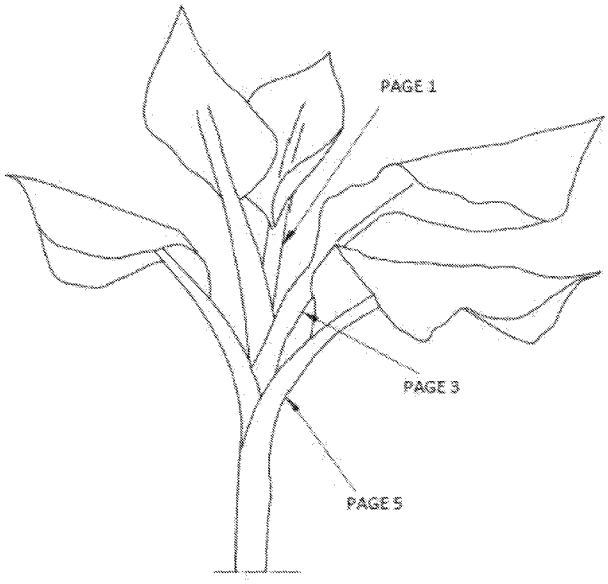


FIG. 1

(57) Abstract: The present invention refers to the use of cinnamaldehyde, preferably in the absence of other essential oils, for the treatment and/or prevention of the black sigatoka fungal disease. The present invention also relates to a composition for the treatment and/or prevention of the black sigatoka fungal disease, characterised in that it essentially comprises at least one active ingredient consisting of cinnamaldehyde. More specifically, the composition may comprise the following components as percentages by weight of the total percentage of the composition: (a) 10 from 1% to 60% of at least one first active ingredient consisting of cinnamaldehyde; (b) from 0.01% to 5% of at least one surfactant substance; and (c) from 10% to 75% of at least one mineral oil. additionally, using the composition for treating the black sigatoka fungal disease is also the object of the invention, which is characterised in that said composition is administered on a banana crop infected by said disease.

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# COMPOSITION FOR THE TREATMENT AND/OR PREVENTION OF THE BLACK SIGATOKA FUNGAL DISEASE IN BANANA CROPS

## DESCRIPTION

### TECHNICAL FIELD OF THE INVENTION

The present invention refers to the use of cinnamaldehyde for controlling the diseases that afflict banana crops. More particularly, the present invention also refers to a new formulation for controlling the diseases that afflict banana crops in plantations, and more specifically to the group of fungal diseases caused by the *Mycosphaerella fijiensis* fungus, more commonly known as *Black Sigatoka*. It is therefore an effective alternative to synthesised chemical fungicides for treating this type of diseases, in order to avoid or reduce the use of said chemicals.

### BACKGROUND OF THE INVENTION

Black Sigatoka is caused by the *Mycosphaerella fijiensis* fungus and is considered the biggest leaf disease affecting banana and plantain crops all around the world, causing losses of up to 100% of the crop (Alejandra Cuéllar Quintero et al., Rev. Fac. Nal. Agr. Medellin 64(1): 5853-5865. 2011). According to the authors mentioned above, this disease causes serious issues for the plant's growth, which suffers severe deterioration in the area of the leaf, affecting the productivity of the crop since it decreases its capacity to photosynthesise. Therefore, the plant is not able to get the necessary nutrients it needs from the leaves in order to feed the bunch of bananas. In consequence, the fruit ripens prematurely, which makes it unsuitable for export. This causes large economic losses.

The same publication also describes how the means used to control Black Sigatoka have been traditionally based on using chemicals that, although they are effective against the disease, they also have a series of disadvantages resulting from their damaging effects on the environment. In addition, the pathogen populations develop resistances to certain systemic fungicides used frequently, such as for example, benzimidazoles, and more recently, triazoles.

Bennet, R.S. and Arneson P.A. describe in their "Black Sigatoka" publication of 2003 (translated to Spanish by Robert J. Knight, Jr., 2005, "The Plant Health Instructor", DOI:10.1094/PHI-I-2005-0217-01) that large banana plantations base most of their

control programs on using protective fungicides, such as for example mancozeb or chlorothalonil. In the case of mancozeb, it is usually applied in combination or alternatively with morpholine, demethylation inhibitors (DMIs), or strobilurin fungicides (QoIs), with diseases usually showing resistances in many areas where bananas are grown.

In addition, it is also possible to find in patent literature some examples of inventions related to the use of chemical fungicides for the treatment of Sigatoka.

For example, patent application US2013296374 describes a new process for controlling Black Sigatoka using agrochemical products, such as for example UK-2A profungicides.

Additionally, application CN104823981 describes a bactericide composition comprising isopyrazam and fludioxonil, which can be used to treat and prevent Sigatoka.

The application CN102578129 also describes a bactericide composition to control Sigatoka characterised in that it comprises difenoconazole and prochloraz.

Finally, application GB2288980 describes a method for preventing Black Sigatoka by using fenpropidin, which belongs to the chemical group of piperidines.

All the aforementioned inventions are based on using chemical agents. However, in spite of their effectiveness, said compositions have a series of drawbacks that makes it convenient to replace them for new alternatives. In this regard, in addition to the financial cost involved in using them, their significant environmental impact should also be taken into account. Moreover, the repeated use of said compositions with the aim of getting the best results in controlling Black Sigatoka has caused in the last years an increase in the number of resistances to certain types of fungicides, which in turn significantly reduces their effectiveness in controlling and treating the disease.

The present invention is framed therefore within the current existing policies in the areas of growing bananas, which are aimed at reducing the use of chemical fungicides. This situation is worsened due to the fact that the areas and countries where bananas are grown are usually tropical and/or subtropical countries, which are noted for being very sensible ecosystems.

Up to now, the development of new biological control methods for Black Sigatoka has been very limited since chemical controls, in addition to being highly effective and cheap, are widely available to producers.

It is therefore the goal of the invention to present a-more environmentally friendly approach as compared to commercially available chemical fungicides with the aim of

controlling the diseases afflicting banana crops in plantations, and more specifically to the group of fungus diseases caused by the *Mycosphaerella fijiensis* fungus, commonly known as Sigatoka or Black Sigatoka.

The only previous invention found to date based on using essential oils for treating Black Sigatoka is the international patent WO 2014/153042. However, unlike the present invention, the composition described in said document is based on a mixture of essential oils, all of which are used in very low concentrations (lower than 5% by weight). In addition, the fungicide composition uses a vegetable oil (preferably palm oil) as solvent, in a proportion that can account for up to 80% by weight of the composition. As a result of the high concentration of oils, which are characterised for oxidising easily, the composition requires using antioxidants. In addition, it also requires using a thickener (Xanthan gum) and an emulsifying agent (wetting agent) in a proportion below 20% by weight. Moreover, the product is used directly for treating the crops, applying it in doses between 2 and 4 litres per hectare. More specifically, cinnamon oil is used in a proportion of 0.5% to 2.5% of the weight of the composition, so that the quantity of said compound is minimal in comparison with the quantity used according to the present invention or in the formulation described in the present invention. In addition, unlike the composition described in WO 2014/153042, the composition described in the present invention has the advantage that it does not use any antioxidants.

WO'042 primarily discloses a method of treating *Black Sigatoka* fungus in crops of the *Musaceae* family using a concoction of essential oils consisting of garlic oil, rosemary oil, thyme oil and cinnamon oil. The specification of WO'042 further stipulates that the cinnamon oil used is distilled from the cinnamon bark of *Cinnamomum zeylanicum*. Paragraph [0011] of WO'042 discloses the concentration, by percentage weight, of the composition before dilution for application as comprising only about 0.5% - 2.5% of cinnamon oil, and preferably only about 0.5% of cinnamon oil. This disclosure does not envisage any beneficial property of cinnamon oil alone, free of other essential oils, for the use as envisaged by the present invention.

## DESCRIPTION OF THE INVENTION

The term 'disease control' as used herein denotes control and prevention of a disease. The term 'cinnamon aldehyde' can be used interchangeably with cinnamaldehyde. Controlling effects include all deviation from natural development, for example: killing, retardation, decrease of the fugal disease. The term 'plants' refers to all

physical parts of a plant, including seeds, seedlings, saplings, roots, tubers, stems, stalks, foliage and fruits. The 'locus' is intended to include soil, seeds, and seedlings as well as established vegetation. Banana plants may be of any variety of the species Musa x paradisiaca. "Plant extracts" maybe any natural product obtained from a plant with the aim of treating plant diseases, among other uses that are not mentioned herein for not being the object of the present invention. In general, the extract may be obtained from the plant by means of any known method of extraction, and occasionally of purification, of essential oils, such as for example, distillation (preferably straight steam distillation), filtration, precipitation, drying, etc.

The term "consisting essentially of" is meant to include the main elements, namely, cinnamaldehyde which may form at least 5% of the total weight of the composition, and the term "substantially free of" is meant to indicate a composition or combination or formulation that contains less than 0.1% of other essential oils. It will be apparent to those skilled in the art that embodiments of the invention, other than those specifically described for illustrative purposes, may be evolved and that modifications in these specific embodiments may be made without departing from the spirit and scope of the invention as expressed above and as defined in the appended claims.

Therefore, one object of the present invention is to provide the use of cinnamaldehyde for the treatment and/or prevention of the Black Sigatoka fungal disease in a banana crop infected by said disease or potentially infected by it. Preferably, in this use, cinnamaldehyde is used alone as the active ingredient in the absence of any other essential oil.

Another object of the invention is a composition for the treatment and/or prevention of the Black Sigatoka fungal disease in a banana crop infected by said disease or potentially infected by it, characterised in that it comprises at least one active ingredient consisting of cinnamaldehyde. Preferably, this composition comprises at least about 5% of cinnamaldehyde by weight of the composition, and is totally free of other essential oils.

In this aspect, the present invention presents a composition for controlling and/or preventing Black Sigatoka in banana, characterised in that it consists essentially of cinnamaldehyde as the active ingredient.

In another embodiment, the composition of the invention comprises:

a first active ingredient consisting essentially of cinnamaldehyde;

at least one surfactant; and

at least one mineral oil.

In an embodiment, the composition of the invention comprises cinnamaldehyde in an amount of from about 5% to about 60% by weight, preferably from about 10% to about 25% of the total composition by weight.

The surfactant may be selected from known surfactants that are added to such formulations according to the practices in the art. The surfactant may be selected from anionic, non-ionic, ionic or amphoteric surfactants. The concentration of the surfactant may be 0.01 to 1%, preferably between 0.01 to 0.03% of the total weight of the composition.

In an embodiment, the composition of the invention may additionally include mineral oil. The mineral oil of the present invention, may be the ones commonly used in combination with synthesised chemical fungicides available in the state of the art. Said mineral oils are characterised for being effective by themselves in treatments against Sigatoka, since they produce the physical effect of covering the leaves. In addition, the physical and chemical properties of vegetable extracts, generally lipophilic oils, make them suitably compatible with mineral oils. For this reason, using mineral oil in the composition achieves a special synergy for the treatment against Sigatoka. Although the mineral oil selected can be any of the ones commonly used in the field, the preferred embodiment uses at least one mineral oil based on distilled hydrocarbons and light paraffinic oils (such as for example, mineral oil CAS No. 64742-56-9). The mineral oil may be added preferably in the concentration of 10 to 75% of the total weight of the composition, most preferably 30 to 50 % of the total weight of the composition.

In some of the embodiments of the invention and with the goal of reducing costs, the active ingredients derived from plant extracts may also be obtained through a process of synthesis. In the specific case of cinnamaldehyde, said active ingredient may consist of a natural extract derived from cinnamon or it may consist of synthesised cinnamaldehyde, used in industrial quantities as a perfume or flavouring agent. If synthesised cinnamaldehyde is used, it will have all the advantages of the claimed composition at a lower cost, similar to that of currently available synthesised fungicides, making it more accessible to the final user.

In short, the main advantages of the invention are the following:

1. Firstly, it is new and environmentally safe solution that is not based on synthesised chemical fungicides, which is especially efficient for controlling Black

Sigatoka in banana crops, showing acceptable effectiveness levels, no phytotoxicity and high biodegradability;

2. The present invention may eliminate or at least reduce in large quantities the massive use of synthesised chemical fungicides, which incur a large financial cost, and above all have a significant environmental impact. Said environmental impact is worsened by the fact that the areas affected by Black Sigatoka are mostly tropical areas with very precious yet also fragile ecosystems. This policy to reduce the use of synthesised chemical fungicides is being promoted with the aim of protecting the environment in countries that grow bananas, since these compositions are especially polluting. The present invention gives the possibility of using it alternatively with said chemical treatments, replacing them or at least reducing their usage. In addition, the new use of cinnamaldehyde and the composition comprising cinnamaldehyde can be used alongside said conventional chemical treatments, preferably in lower doses;

3. Finally, the composition of the invention is a stable formulation that is compatible with the use of mineral oils commonly used in treatments for controlling banana diseases, which is free from antioxidants.

Additionally, using cinnamaldehyde and its composition according to the present invention for treating and/or preventing the Black Sigatoka fungal disease is also the object of the invention, which consists of applying cinnamaldehyde, or alternately the composition described above, on a banana crop infected by said disease. The composition can be applied using the same conventional system for applying treatments based on synthesised chemical fungicides on banana crops (plantation) during preharvest, preferably by means of spraying or fumigating the field (land or aerial).

## DETAILED DESCRIPTION OF THE INVENTION

Therefore, in one aspect, the present invention provides the use of cinnamaldehyde for the treatment and/or prevention of the Black Sigatoka fungal disease in a banana crop infected by said disease or potentially infected by it. Preferably, in this use, cinnamaldehyde is used alone as the active ingredient in the absence of any other essential oil.

In a non-limiting embodiment, cinnamaldehyde is administered in the form of a composition.

Given the physical and chemical characteristics of plant extracts and with the aim of achieving compatibility between the composition that is the object of the invention with at least one mineral oil in the proportions preferred by the invention, the composition may be an emulsifiable concentrate.

Therefore, in aspect of the present invention cinnamaldehyde present in the composition may be used as part of a liquid solution that may comprise, as a percentage by weight of the total percentage of the solution:

- from 10% to 70% of the active ingredient;
- from 10% to 80% (of at least one emulsifying substance, selected from a nonionic emulsifier and more preferably an emulsifier selected from a dioleate polyethylene glycol (PEG) 400 (CAS 25322-68-3), Pluronic® (copolymer of ethylene oxide-propylene oxide) and polysorbate 80, or combinations thereof.; and
- from 5% to 20% (and preferably 10%) of at least one solvent, preferably propylene glycol.

In an embodiment the cinnamaldehyde is 30% of the total weight of the formulation.

In an embodiment, the emulsifying agent is 50 -70% of the total weight of the formulation.

In a preferred embodiments of the invention, this liquid solution of the active ingredient can comprise the following components as percentages by weight of the total percentage of the solution;

- 30% of cinnamaldehyde;
- 10% of polysorbate 80;
- 10% of Pluronic® (copolymer of ethylene oxide-propylene oxide)
- 40% of PEG 400 and
- 10% of propylene glycol.

In another embodiment of the invention, the liquid solution of the active ingredient can comprise the following components as percentages by weight of the total percentage of the solution;

- 30% of cinnamaldehyde;

- 20% of polysorbate 80;
- 40% of PEG 400 and
- 10% of propylene glycol.

One of the advantages of the composition of the present invention is that it is compatible with synthesised chemical fungicides commonly used for controlling Sigatoka. Therefore, in the embodiments of the invention, cinnamaldehyde and/or the composition can be used in combination with synthetic agrochemicals as well organic actives known to be used in the control of Black Sigatoka, which creates a synergy that improves the effect achieved by using synthesised chemical fungicides, or it can also be used to control the appearance of strands that are resistant to said synthetic fungicides. This way, in the embodiments of the invention, cinnamaldehyde and/or the composition may be used in combination with at least one synthesised chemical fungicide, or it may be used alternatively with other treatments, in which case a series of treatments based on the composition of the invention would be applied followed by a series of treatments based on at least one synthesised chemical fungicide. Preferably, the synthesised chemical fungicide will be selected from among the fungicides authorised for controlling Black Sigatoka in banana crops such as those fungicides belonging to dithiocarbamates, carbamates, aromatic fungicides, benzimidazoles, strobilurins, conazoles, amines, anilinopyrimidines, succinate dehydrogenase inhibitors and combinations thereof.

In an embodiment, cinnamaldehyde may be used in conjunction with, and the composition of the invention may additionally comprise, a dithiocarbamate fungicide.

The dithiocarbamates may be selected from amobam, asomate, azithiram, carbamorph, Cufraneb, disulfiram, cuprobam, ferbam, metam, nabam, tecoram, thiram, urbacide, ziram, etem, milneb, mancozeb, maneb, metiram, polycarbamate, propineb, zineb and combinations thereof.

In an embodiment, cinnamaldehyde may be used in conjunction with, and the composition may additionally comprise, a carbamate fungicide.

The carbamates may be selected from benthiavalicarb, furophanate, iodocarb, iprovalicarb, picarbutrazox, propamocarb and its derivatives, pyribencarb, thiophanate thiophanate-methyl, tolprocarb, albendazole, benomyl, carbendazim, cypendazole, debacarb, mecarbinzid, diethofencarb, triclopyricarb and combinations thereof.

In an embodiment, cinnamaldehyde may be used in conjunction with, and the composition may additionally comprise, an aromatic fungicide.

In an embodiment, the aromatic fungicide is chlorothalonil.

In an embodiment, cinnamaldehyde may be used in conjunction with, and the composition may additionally comprise, a benzimidazole fungicide.

The benzimidazole fungicide may be selected from albendazole, benomyl, carbendazim, chlorfenazole, cypendazole, debacarb, fuberidazole, mecarbinzid, rabenzazole, thiabendazole, furophanate, thiophanate, thiophanate-methyl and combinations thereof.

In an embodiment, cinnamaldehyde may be used in conjunction with, and the composition may additionally comprise, a strobilurin fungicide.

The strobilurin fungicide may be selected from fluoxastrobin, mandestrobin, azoxystrobin, bifujunzhi,coumoxystrobin, enoxastrobin, flufenoxystrobin, jiaxiangjunzhi ,picoxystrobin ,pyraoxystrobin, pyraclostrobin pyrametostrobin , triclopyricarb, dimoxystrobin, fenaminstrobin, metominostrobin, orysastrobin, kresoxim-methyl, trifloxystrobin.

In an embodiment, cinnamaldehyde may be used in conjunction with, and the composition may additionally comprise, a conazole fungicide.

The conazole fungicide may be selected from Climbazole, clotrimazole, imazalil, oxpoconazole, prochloraz, triflumizole, azaconazole, bromuconazole, cyproconazole, diclobutrazol, difenoconazole, diniconazole, diniconazole-M, epoxiconazole, etaconazole, fenbuconazole, fluquinconazole, flusilazole, flutriafol, furconazole, furconazole-cis, hexaconazole, imibenconazole, ipconazole, ipfentrifluconazole, mefentrifluconazole, metconazole, myclobutanil, penconazole, Propiconazole, prothioconazole, quinconazole, simeconazole, tebuconazole, tetraconazole, triadimefon, triadimenol, triticonazole, uniconazole, uniconazole-P

In an embodiment, cinnamaldehyde may be used in conjunction with, and the composition may additionally comprise, an amine fungicide.

The amine fungicides may be selected from spiroxamine, fenpropimorph, fenpropidin and tridemorp.

In an embodiment, cinnamaldehyde may be used in conjunction with, and the composition may additionally comprise, an anilinopyrimidine fungicide.

The anilinopyrimidine fungicide is preferably Pyrimethanil.

In an embodiment, cinnamaldehyde may be used in conjunction with, and the composition may additionally comprise, a succinate dehydrogenase inhibitor fungicide.

The succinate dehydrogenase inhibitors may be selected from boscalid, fluopyram, fluxapyroxad and isopyrazam.

Thus in an embodiment, the present invention may provide a composition of cinnamaldehyde and an emulsifier and a synthetic fungicide selected from carbamate, dithiocarbamate, benzene derivatives, benzimidazoles, strobilurins, conazoles, amines, anilinopyrimidines, succinate dehydrogenase inhibitors and combinations thereof.

Using cinnamaldehyde, or the composition of the invention comprising cinnamaldehyde, for the treatment and/or prevention of Black Sigatoka, either by itself or in combination with at least one synthesised chemical fungicide suitable for treating said disease, is therefore an additional object of the invention. In the case when said synthesised chemical fungicide is used in the usual dose (as detailed in the technical data sheet of the product or on its label), using the composition that is the object of the invention will reinforce its effectiveness for the treatment of Sigatoka. If, by contrast, a lower dose than usual of the chemical fungicide was used, the main advantage of the invention in this case would be reducing the amount of said synthesised chemical fungicide used, achieving synergy with the composition of the invention.

Preferably, applying cinnamaldehyde, or the composition of the invention comprising cinnamaldehyde, can be done by following the same frequency of application used in the case of synthesised chemical fungicides (as for example, mancozeb), which is preferably a dose of 2.5 to 6 litres/Ha or once the symptoms of the disease reappear.

Preferably, each of the components of the mixture will be applied as follows:

- Cinnamaldehyde (solution), from 1 to 10 l/Ha;
- Mineral oil, from 1 to 5 l/Ha, preferably 3 l/Ha;

Surfactant (or mixture of surfactants) in quantities equal to the 1% of the mineral oil selected.

Alternatively, the present invention provides a method for controlling/ treating black sigatoka disease in banana which comprises applying to the foliage of the plants a fungicidally effective amount of cinnamaldehyde, or a composition comprising cinnamaldehyde.

In an embodiment, the composition may comprise at least one chemical fungicide.

In another embodiment, the method of the present invention may provide a composition comprising cinnamaldehyde, and at least one chemical fungicide.

In an embodiment, the composition of the present invention can be packaged as a kit-of-parts. In an embodiment a kit-of-parts may contain various components of the formulation of the present invention that may can be mixed as instructed prior to spraying.

Accordingly, one aspect of the present invention can provide a multi-pack fungicidal product for controlling black sigatoka at a locus comprising components of the present invention and a manual. The instruction manual includes instructions for administering the components of the present composition.

In an embodiment, the instruction manual includes instructions for administering the fungicidal composition at a locus.

In another embodiment, the instruction manual includes instructions for admixing the cinnamaldehyde component with the remaining ingredients of the composition or with another chemical fungicide.

In an embodiment, the combination kit is packed in a package or a carton. In another embodiment, the instruction manual may be printed on said package or carton or may be printed on a booklet that may be included within the package or the carton.

**BRIEF DESCRIPCION OF THE FIGURES**

The drawings accompanying this description show the results of the tests carried out to analyse the effectiveness of the composition that is the object of the invention, as described in the example detailed below:

- Figure 1 shows the methodology for counting the leaves of the banana plant (*Musa spp*). As shown in this drawing, counting starts on the top leaves and continues down to the bottom leaves.
- Figure 2 shows the weekly analysis of the youngest leaf infected (YLI) in all the treatments carried out;
- Figure 3 shows the weekly analysis of the youngest leaf spotted (YLS) in all the treatments carried out;
- Figure 4 shows the weekly analysis of the total number of leaves in all the treatments carried out;
- Figure 5 shows the weekly analysis of the severity of the disease for the treatments carried out using Formulation 1;

  Figure 6 shows the weekly analysis of the severity of the disease for all the treatments carried out.

**Example: Comparative test of the effectiveness of the composition that is the object of the invention and a synthesised chemical fungicide (more specifically, the fungicide Mancozeb)**

An example is described below with the goal of proving the effectiveness of the composition of the invention for the treatment of Black Sigatoka in banana crops. This example shows the results of a series of effectiveness tests carried out in Rita, Guapiles (Costa Rica).

*Grande Naine Tissue* banana plants were used for the test. An experimental unit consisted of 10 banana plants planted in a line at a distance of 2.5 meters between each plant.

The test design was completely random and each treatment was repeated three times. *Musa textilis*, a variety of plant that is resistant to Black Sigatoka, was planted on the rows separating the treatments.

Every treatment was applied using a motor sprayer for a volume of cinnamaldehyde formulation of 2.5 l/Ha. The volumes of the tested products were mixed in a mineral oil emulsion in a dose of 3 l/Ha of mineral oil with 0.03/Ha of surfactant (1% of the mineral oil used).

More specifically, the following formulations were used in the treatment:

Table 1. Formulation 1

| Percentage (weight) | |
|---|---|
| 45.26% | Cinnamaldehyde solution comprised of (percentage by weight of the total percentage of the composition):<br>- 30% of cinnamaldehyde;<br>- 10% of polysorbate 80;<br>- 10% of Pluronic®;<br>- 40% of PEG 400 and<br>- 10% of propylene glycol. |
| 0.54% | Imbirex® (mixture of nonionic surfactants) |
| 54.2% | Mineral oil (CAS No. 64742-56-9) |

Table 3 shows the compositions used for each treatment, and the quantities used in each of the compositions:

Table 2. Treatments

| | Formulation | Amount applied (l/Ha) | Active ingredient |
|---|---|---|---|
| 1 | Formulation 1 | 1.5 | Cinnamaldehyde |
| 2 | Formulation 1 | 2.5 | Cinnamaldehyde |
| 3 | Formulation 1 | 6.0 | Cinnamaldehyde |
| 4 | Vondozeb® (Fungicide) | 2.0 | Mancozeb |
| 5 | Spraytex® | 3.0 | Mineral oil |
| 6 | Untreated plant | - | - |

The treatments were first applied on May 20, 2015 (week 21), and on August 5, 2015 (week 32) for the final time. They were applied a total of 14 times.

The following variables were analysed on a weekly basis: total number of leaves per plant, youngest leaf infected (YLI), youngest leaf spotted (YLS), and the severity of the disease. The studies of the disease were carried out once the first leaf treated reached the position 4, and every 7 days for up to two weeks after the last instance when the formulations were applied.

The Black Sigatoka's response to the treatment was evaluated using the Stover scale modified by Gauhl (Table 4).

Table 3.

| Degree | Description |
|---|---|
| 0 | Absence of symptoms |
| 1 | Presence of streaks up to 10 spots |
| 2 | 11 spots on up to 5% of the area of the leaf |
| 3 | Spots on from 6% to 15% of the area of the leaf |
| 4 | Spots on from 16% to 33% of the area of the leaf |
| 5 | Spots on from 34% to 50% of the area of the leaf |
| 6 | Spots on over 50% of the area of the leaf |

Statistical analysis was carried out using the INFOSTAT program, using a multiple comparison test (Tukey) for each parameter (Table 5.1)

The parameters evaluated for the statistical analysis were transformed as area under the curve (AUC). AUC is the mathematical operation representing the addition of

each of the parameters analysed between two testing dates, multiplied by the days between the two tests (X week 21 + X week 22)/2 *(weekdays 21-22).

Table 5.1 shows the AUC values used only for statistical analysis:

Table 4.1

| Formulation | YLI Pr 0.0165 | YLS Pr 0.8030 | Number of leaves Pr 0.9908 | Severity Pr 0.0202 |
|---|---|---|---|---|
| Formulation 1 | 258.2 AB | 288.5 A | 489.5 A | 87.3 AB |
| Formulation 1 | 262.0 AB | 309.7 A | 484.5 A | 68.5 AB |
| Formulation 1 | 271.6 AB | 313.5 A | 498.0 A | 67.0 AB |
| Vondozeb® 62 SC | 312.0 B | 331.3 A | 489.0 A | 35.4 A |
| Spraytex® | 248.7 A | 304.8 A | 494.0 A | 97.5 B |
| Untreated plant | 242.2 A | 314.8 A | 493.0 A | 108.2 B |

*Tukey:* Mean with the same letter is not statistically different (P=0.05).

Table 5.2 shows the mean result of 10 tests (not transformed to AUC):

Table 5.2.

| Formulation | YLI Pr 0.0165 | YLS Pr 0.8030 | Number of leaves Pr 0.9908 | Severity Pr 0.0202 |
|---|---|---|---|---|
| Formulation 1 | 4.2 | 5.1 | 7.9 | 1.43 |
| Formulation 1 | 4.2 | 5.2 | 7.8 | 1.15 |
| Formulation 1 | 4.4 | 5.3 | 8.0 | 1.10 |
| Vondozeb® 62 SC | 5.0 | 5.6 | 7.9 | 0.62 |
| Spraytex® | 4.0 | 5.1 | 7.9 | 1.60 |
| Untreated plant | 3.9 | 5.1 | 7.9 | 1.76 |

The figures accompanying this description show the results of the tests carried out.

It should be taken into account that the first appearance of symptoms is correlated with the severity of the infection. Therefore, the lower the number is of leaves on which the symptoms appear, the higher the infection level.

In all the drawings, axis X represents the week, and axis Y represents YLI, YLS, the total number of leaves or the severity index of the disease.

Results

The following conclusions can be drawn from the results of the tests:

- Firstly, a similar control was observed in all the treatments when the YLI was analysed (the youngest leaf with streaks). In all the cases, the values were in the range of 3.5-5.0 leaves during most of the test (see figure 2). According to the statistical analysis, there were no significant differences for YLI between the different types of formulations. However, there were differences between the treatment with Vondozeb®, the case of untreated plants and the treatment with a mineral oil (see Table 5.1);
- A similar trend was observed for the YLS (youngest leaf spotted) parameter, since similar results were obtained using Formulation 1. However, the treatment with Vondozeb (Mancozeb) had a notably lower impact on the disease (higher values of YLS) in comparison with the rest of the fungicides (see Table 5.2 or figure 3). In this case, no statistical analysis was carried out since the probability that the YLS values reflected any kind of effect was very low (P= 0.80).
- As shown in figure 4, the total number of leaves ranged between 5.0 and 11.0 during the testing period. The behaviour was very similar between all the treatments tested, including the tests with Vondozeb® and the mineral oil. Due to the high number of infections in the case of untreated plants, their graphs had a slightly lower number of leaves in the last three tests. The statistical treatment shows that none of the treatments had any effect on the number of leaves (P=0.99);
- As for figures 5 to 7, it should be highlighted that the severity of the disease is the most reliable parameter to compare the differences between the treatments. The results show that the Formulation 1 is active against Black Sigatoka since the medium dose (2.5 l/Ha) and the higher dose (6.0 l/Ha) were significantly more effective than the mineral oil (see figure 5). Although the mineral oil does not have in and of itself any fungicidal effect, it helps the fungicide to spread and also to penetrate the surface of the leaf.

The previous results show the effectiveness of the composition that is the object of the invention, which therefore proves to be an effective alternative for replacing synthesised chemical fungicides.

## CLAIMS

1. Use of cinnamaldehyde for the treatment and/or prevention of Black Sigatoka fungal disease.

2. Use as claimed in claim 1 characterized in that cinnamaldehyde is used totally free of other essential oils.

3. Use as claimed in claim 1 or claim 2, in combination with a second fungicide.

4. Use as claimed in claim 3, wherein said second fungicide is selected from:

(a) a dithiocarbamate fungicide selected from amobam, asomate, azithiram, carbamorph, Cufraneb, disulfiram, cuprobam, ferbam, metam, nabam, tecoram, thiram, urbacide, ziram, etem, milneb, mancozeb, maneb, metiram, polycarbamate, propineb, zineb and combinations thereof; or

(b) a carbamate fungicide selected from benthiavalicarb, furophanate, iodocarb, iprovalicarb, picarbutrazox, propamocarb and its derivatives, pyribencarb, thiophanate, thiophanate-methyl, tolprocarb, albendazole, benomyl, carbendazim, cypendazole, debacarb, mecarbinzid, diethofencarb, triclopyricarb and combinations thereof; or

(c) chlorothalonil; or

(d) a benzimidazole fungicide selected from albendazole, benomyl, carbendazim, chlorfenazole, cypendazole, debacarb, fuberidazole, mecarbinzid, rabenzazole, thiabendazole, furophanate, thiophanate, thiophanate-methyl and combinations thereof; or

(e) a strobilurin fungicide selected from fluoxastrobin, mandestrobin, azoxystrobin, bifujunzhi,coumoxystrobin, enoxastrobin, flufenoxystrobin, jiaxiangjunzhi, picoxystrobin, pyraoxystrobin, pyraclostrobin pyrametostrobin, triclopyricarb, dimoxystrobin, fenaminstrobin, metominostrobin, orysastrobin, kresoxim-methyl, trifloxystrobin and combinations thereof; or

(f) a conazole fungicide selected from Climbazole, clotrimazole, imazalil, oxpoconazole, prochloraz, triflumizole, azaconazole, bromuconazole, cyproconazole, diclobutrazol, difenoconazole, diniconazole, diniconazole-M, epoxiconazole, etaconazole, fenbuconazole, fluquinconazole, flusilazole, flutriafol, furconazole, furconazole-cis, hexaconazole, imibenconazole, ipconazole, ipfentrifluconazole,

mefentrifluconazole, metconazole, myclobutanil, penconazole, Propiconazole, prothioconazole, quinconazole, simeconazole, tebuconazole, tetraconazole, triadimefon, triadimenol, triticonazole, uniconazole, uniconazole-P and combinations thereof; or

(g) an amine fungicide selected from spiroxamine, fenpropimorph, fenpropidin, tridemorp and combinations thereof; or

(h) Pyrimethanil; or

(i) a succinate dehydrogenase inhibitor fungicide selected from boscalid, fluopyram, fluxapyroxad and isopyrazam.

5. Use as claimed in any one of the preceding claims comprising treating and/or preventing Black Sigatoka fungal disease in banana.

6. Composition for the treatment and/or prevention of the Black Sigatoka fungal disease, characterised in that it comprises at least one active ingredient consisting of cinnamaldehyde.

7. Composition according to claim 6, characterized in that it comprises the following components as percentages by weight of the total percentage of the composition:

(a) from 1% to 60% of cinnamaldehyde;

(b) from 0.01% to 5% of at least one surfactant substance; and

(c) from 10% to 75% of at least one mineral oil.

8. Composition according to claim 7, wherein the cinnamaldehyde is contained in a solution comprising the following components as percentages by weight of the total percentage of the solution:

(a) from 10% to 70% cinnamaldehyde;

(b) from 10% to 80% of at least one emulsifying substance;

(c) from 5% to 20% of at least one solvent.

9. Composition according to claim 8, wherein the emulsifier is selected from a group consisting of dioleate polyethylene glycol (PEG) 400, a copolymer of ethylene oxide-propylene oxide, polysorbate 80, and any combination thereof.

10. Composition according to claims 8 or 9, wherein the solvent consists of propylene glycol.

11. Composition according to claim 6, comprising a second active ingredient of synthetic origin.

12. Composition according to claim 11 wherein the proportion of the second active ingredient ranges between 0.5% and 70% by weight of the total percentage of the composition.

13. Composition according to claim 11, wherein the second active ingredient is a fungicide of the family dithiocarbamates, carbamates, aromatic fungicides, benzimidazoles, strobilurins, conazoles, amines, anilinopyrimidines, succinate dehydrogenase inhibitors or a combination thereof.

14. Method for treating the fungal disease Black Sigatoka, characterized in that it consists of administering a composition comprising at least cinnamaldehyde on a banana crop infected by said disease.

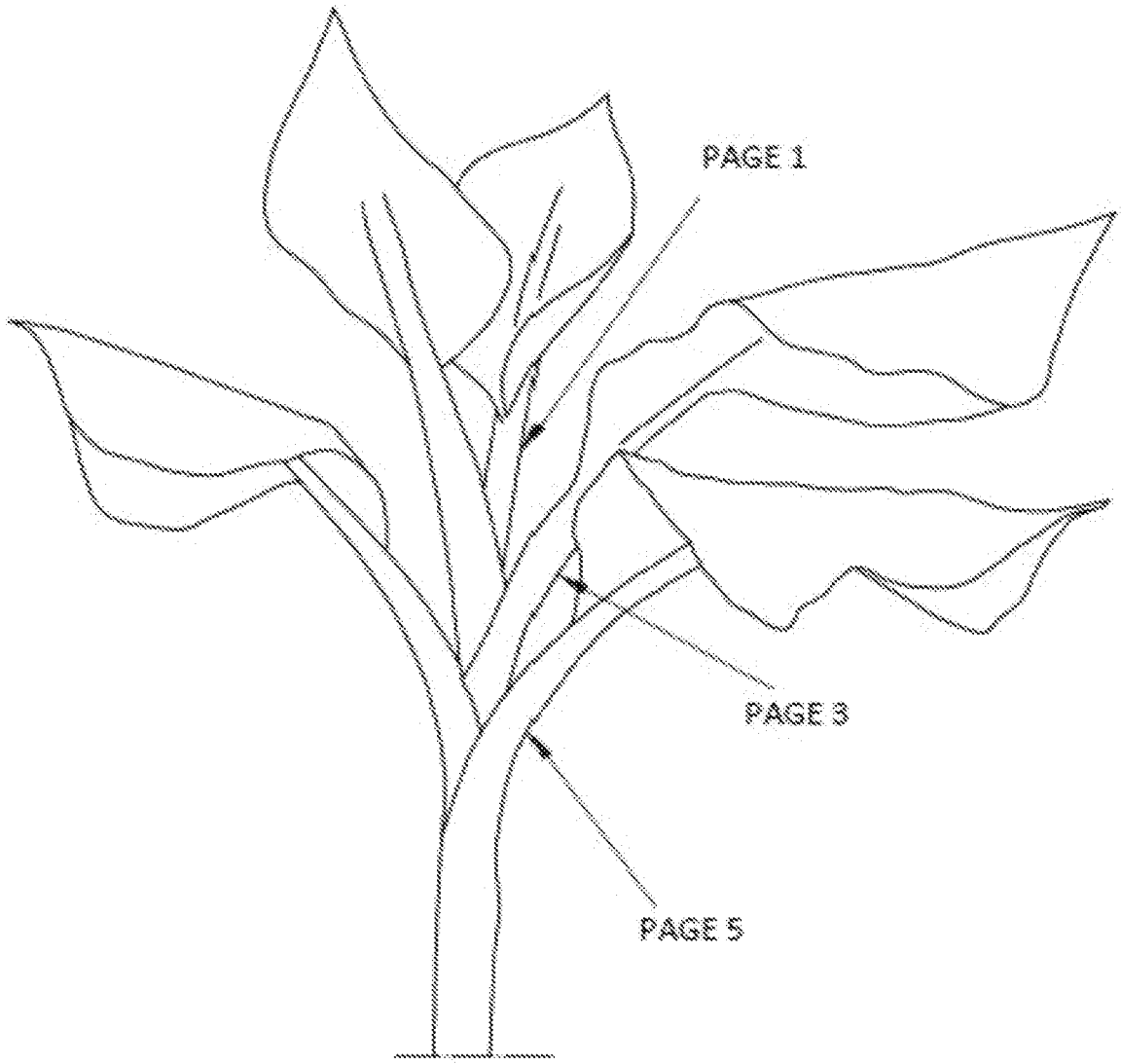
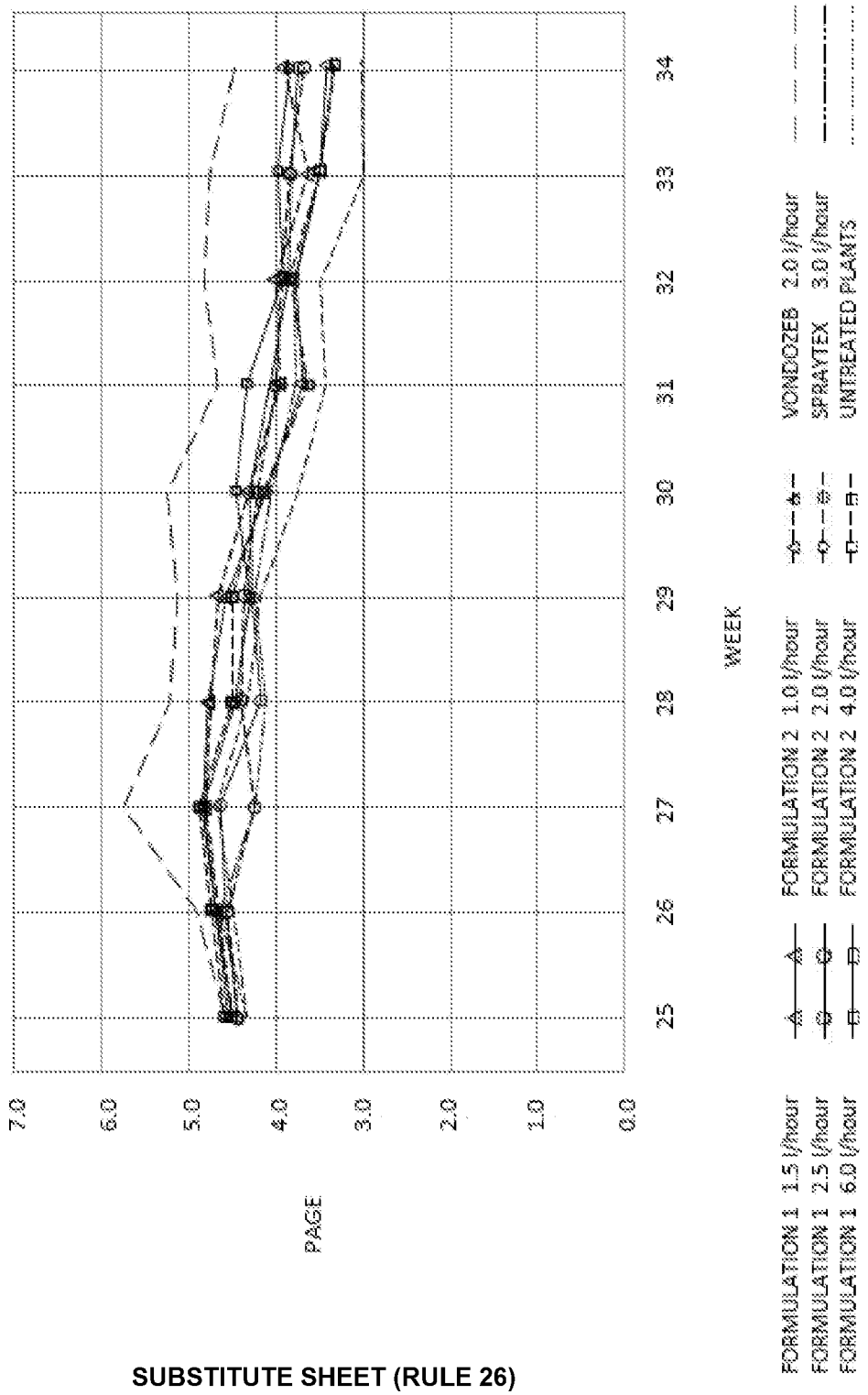



FIG. 1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3.0
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30
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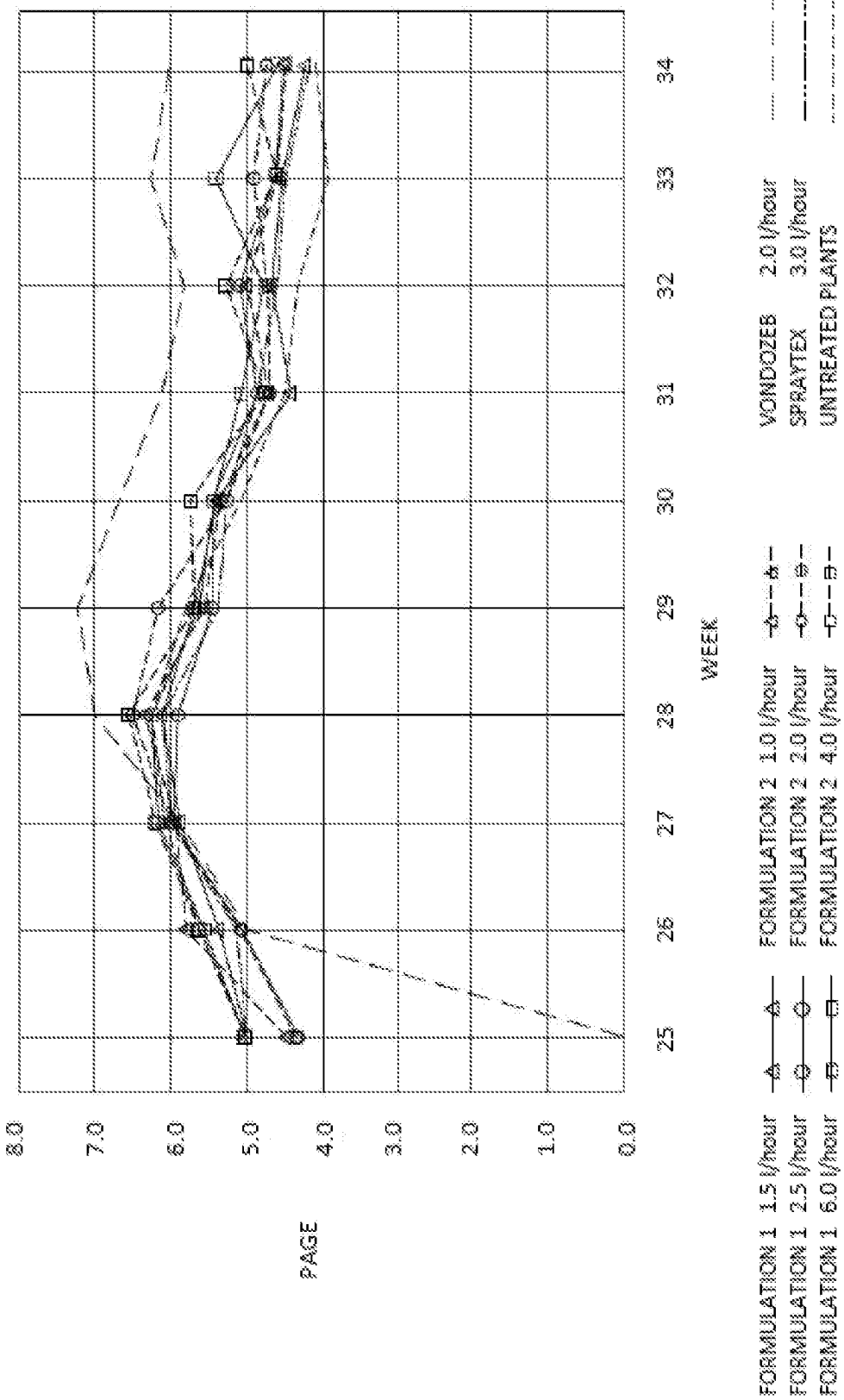
32
33
34
WEEK
FORMULATION 1 1.5 l/hour
FORMULATION 1 2.5 l/hour
FORMULATION 1 6.0 l/hour
FORMULATION 2 1.0 l/hour
FORMULATION 2 2.0 l/hour
FORMULATION 2 4.0 l/hour
VONDOZEB 2.0 l/hour
SPRAYTEX 3.0 l/hour
UNTREATED PLANTS

FIG. 2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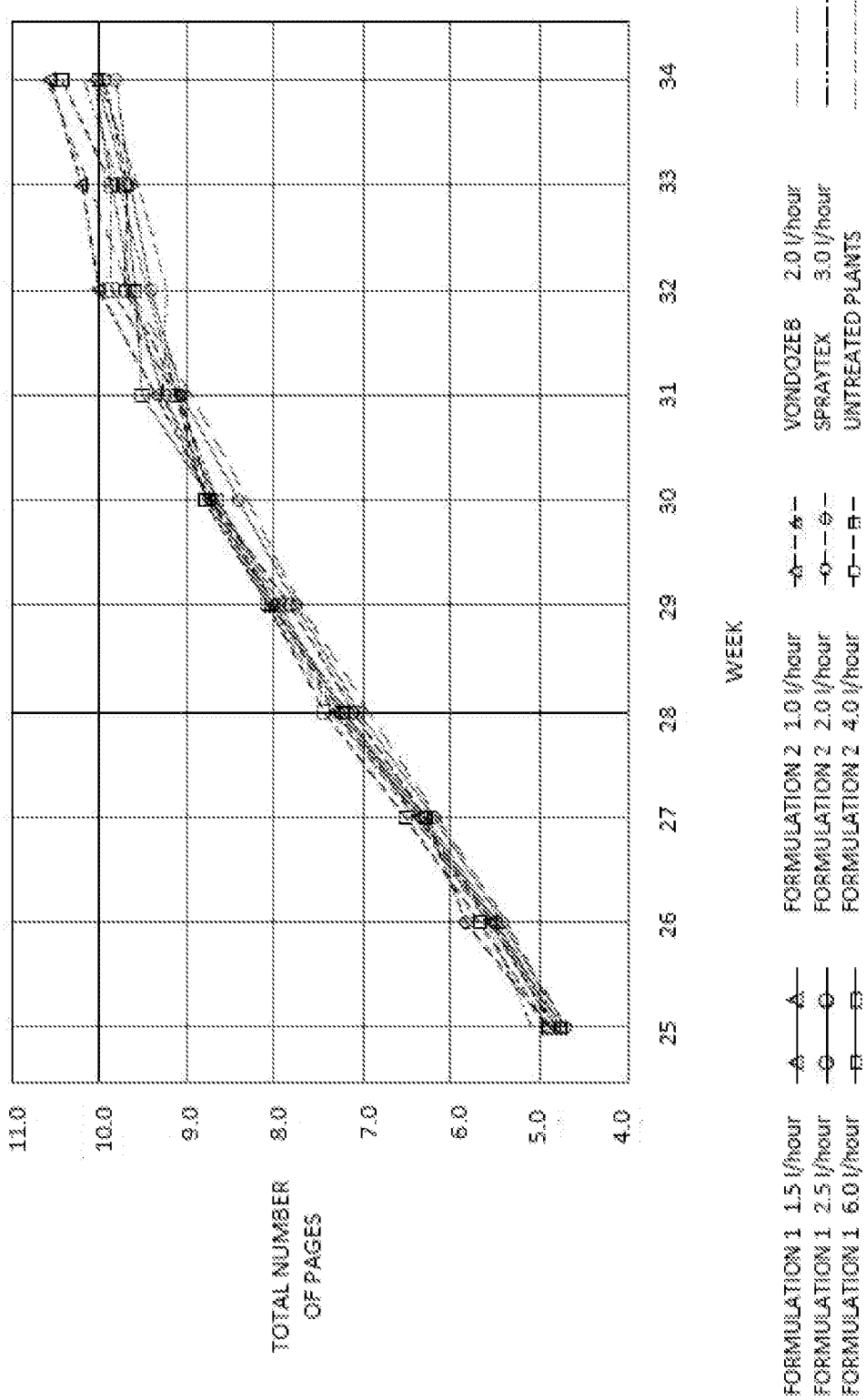
30
31
32
33
34
WEEK
FORMULATION 1 1.5 l/hour
FORMULATION 1 2.5 l/hour
FORMULATION 1 6.0 l/hour
FORMULATION 2 1.0 l/hour
FORMULATION 2 2.0 l/hour
FORMULATION 2 4.0 l/hour
VONDOZEB 2.0 l/hour
SPRAYTEX 3.0 l/hour
UNTREATED PLANTS

FIG. 3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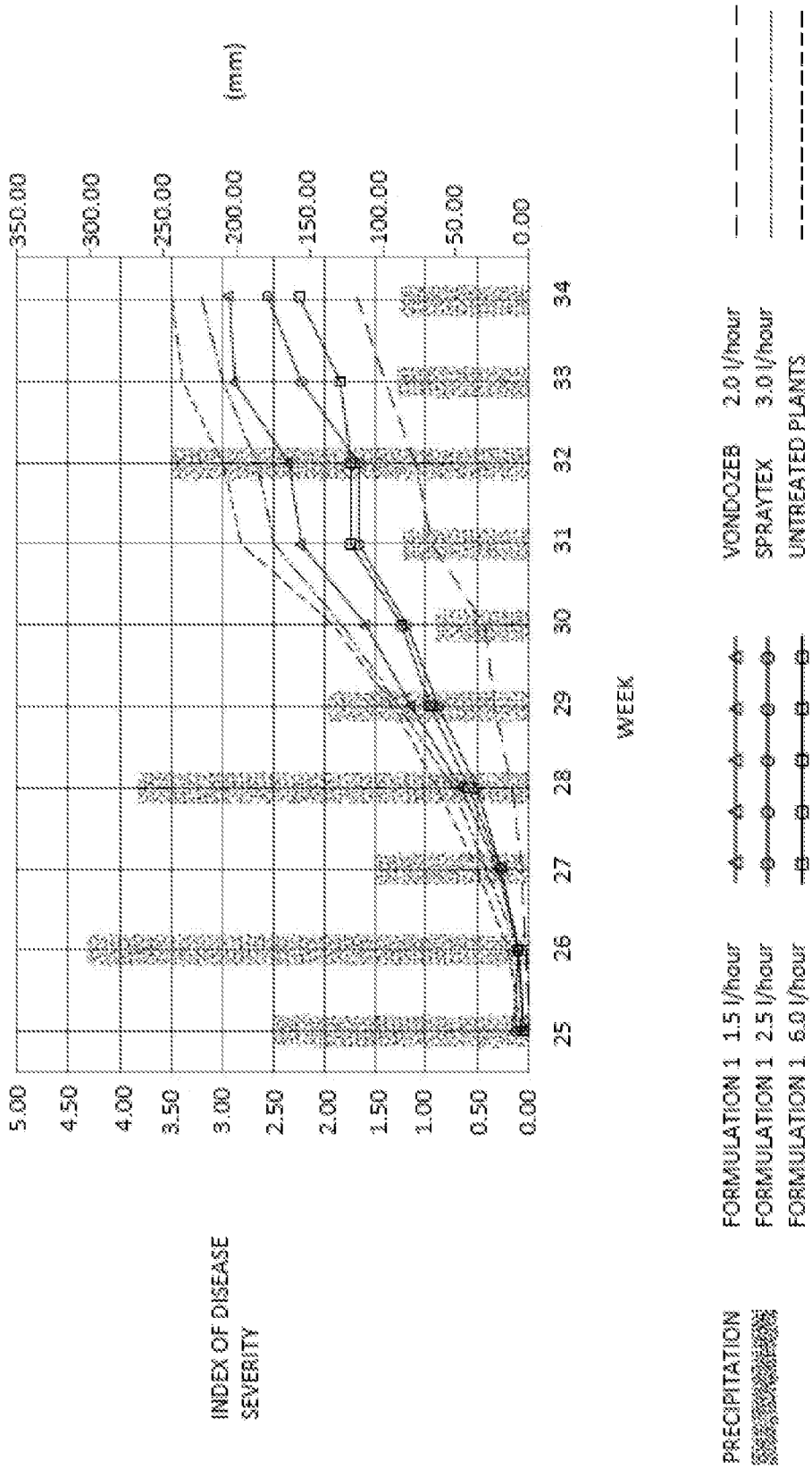
31
32
33
34
WEEK
FORMULATION 1 1.5 l/hour
FORMULATION 1 2.5 l/hour
FORMULATION 1 6.0 l/hour
FORMULATION 2 1.0 l/hour
FORMULATION 2 2.0 l/hour
FORMULATION 2 4.0 l/hour
VONDOZEB 2.0 l/hour
SPRAYTEX 3.0 l/hour
UNTREATED PLANTS

FIG. 4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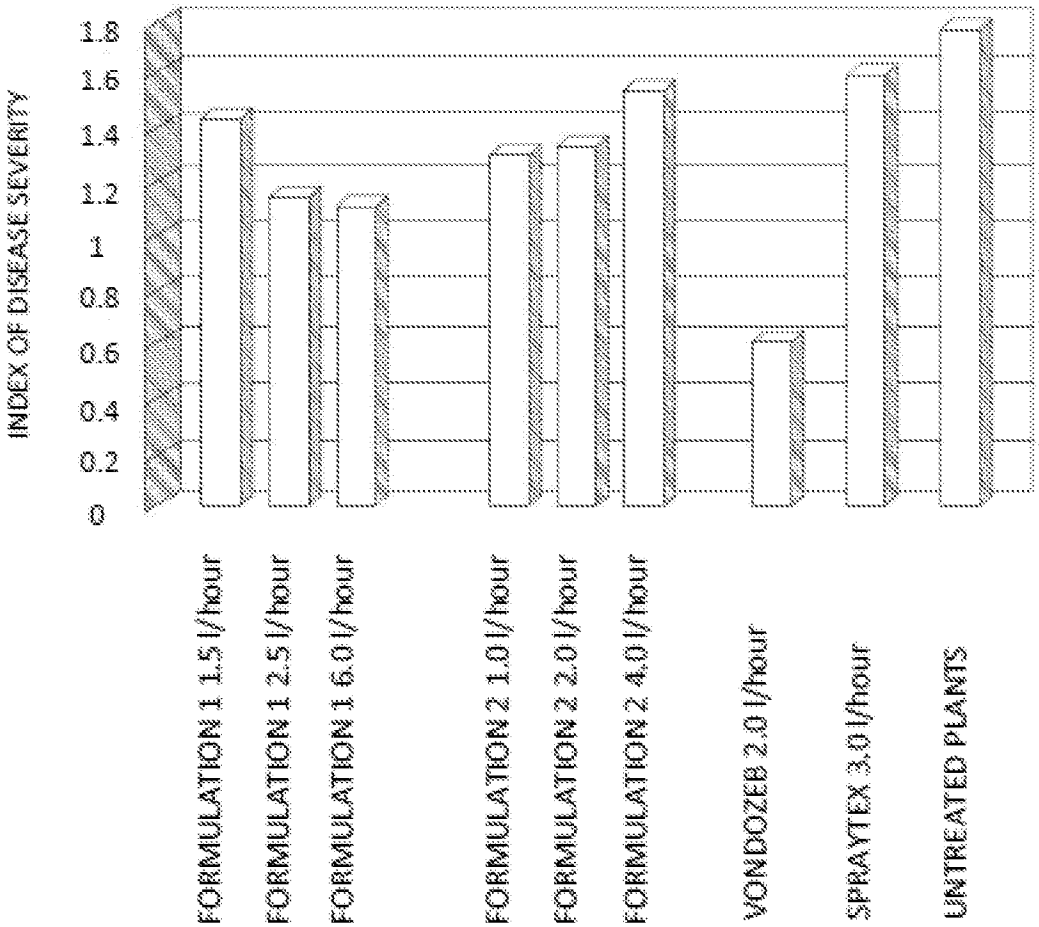
0.00
(mm)
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
25
26
27
28
29
30
31
32
33
34
WEEK
PRECIPITATION
FORMULATION 1 1.5 l/hour
FORMULATION 1 2.5 l/hour
FORMULATION 1 5.0 l/hour
VONDOZEB 2.0 l/hour
SPRAYTEX 3.0 l/hour
UNTREATED PLANTS

FIG. 5


INDEX OF DISEASE SEVERITY
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1.2
1
0.8
0.6
0.4
0.2
0
FORMULATION 1 1.5 l/hour
FORMULATION 1 2.5 l/hour
FORMULATION 1 6.0 l/hour
FORMULATION 2 1.0 l/hour
FORMULATION 2 2.0 l/hour
FORMULATION 2 4.0 l/hour
VONDOZEB 2.0 l/hour
SPRAYTEX 3.0 l/hour
UNTREATED PLANTS

FIG. 6

**INTERNATIONAL SEARCH REPORT**

International application No.
**PCT/IB2017/057241**

A. CLASSIFICATION OF SUBJECT MATTER

***A01N 65/24 (2009.01) A01N 65/00 (2009.01) A01P 3/00 (2006.01) A01N 35/02 (2006.01) A01N 31/04 (2006.01)***

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)

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)

EPOQUENET (Database: Patenw), STN (Databases: CAPLUS, REGISTRY, DWPI, BIOSIS, CAPA), Espacenet, Google Scholar/Patents; Keywords: cinnamon oil/aldehyde, cinnamaldehyde, black sigatoka, black leaf streak, mycosphaerella fijiensis, fungicide, banana, plantain, musaceae, carbamate, chlorothalonil, azole, strobilurin, amine, pyrimethanil, succinate (and similar terms and in combinations); IPC/CPC marks: A01N/A01P; Applicant/Inventors: Decco Worldwide Post-harvest Holdings/UPL Ltd, Akhter SOHAIL, Calderon-Kawasaki KENICHI, Madriz Acevedo GUSTAVO, Gomez Hernandez ENRIQUE, Jaidev Rajnikant SHROFF, Vikram Rajnikant SHROFF; Applicant/Inventor names searched in internal databases provided by IP Australia.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| | Documents are listed in the continuation of Box C | |

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"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 | Date of mailing of the international search report |
|---|---|
| 6 February 2018 | 06 February 2018 |
| **Name and mailing address of the ISA/AU** | **Authorised officer** |
| AUSTRALIAN PATENT OFFICE<br>PO BOX 200, WODEN ACT 2606, AUSTRALIA<br>Email address: pct@ipaustralia.gov.au | Callen Puah<br>AUSTRALIAN PATENT OFFICE<br>(ISO 9001 Quality Certified Service)<br>Telephone No. +61262832209 |

| INTERNATIONAL SEARCH REPORT<br>C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT | | International application No.<br>**PCT/IB2017/057241** |
|---|---|---|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | WO 2014/153042 A1 (GRIFFITH LABORATORIES INTERNATIONAL, INC.) 25 September 2014<br>Abstract, paragraphs [0002], [0006], [0010]-[0012] | 1-14 |
| X<br>Y | Cyclops Organic Fungicide [retrieved from internet on 25 January 2018]<br><URL: http://cru66.cahe.wsu.edu/~picol/pdf/WA/62412.pdf> published on 02 August 2015 as per the index of the parent directory on URL: http://cru66.cahe.wsu.edu/~picol/pdf/WA<br>'Ingredients', 'Manufacturing Under U.S.A and International Patents'<br>'Ingredients', 'Manufacturing Under U.S.A and International Patents' | 1, 2, 7-10<br>3, 4, 11-13 |
| X | WO 2016/154635 A2 (IBEX BIONOMICS, LLC) 29 September 2016<br>Table 8, Example XI and XIII, Figure 55 | 1, 3-14 |
| Y | CN 105145588 A (GUANGXI NANNING YITU BIOTECHNOLOGY CO LTD) 16 December 2015<br>Abstract, paragraphs [0004], [0027] | 3, 4, 11-13 |
| Y | CN 105165856 A (GUANGXI NANNING YITU BIOTECHNOLOGY CO LTD) 23 December 2015<br>Abstract, paragraphs [0019]-[0027] | 3, 4, 11-13 |
| A | SINGH, G., et al., 'A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents', Food and Chemical Toxicology, 2007, vol. 45(9), pages 1650-1661<br>Abstract | 1-14 |
| A | WO 2011/140309 A2 (BIOMOR ISRAEL LTD.) 10 November 2011 | |

| INTERNATIONAL SEARCH REPORT<br>Information on patent family members | International application No.<br>**PCT/IB2017/057241** |
|---|---|

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| **Patent Document/s Cited in Search Report** | | **Patent Family Member/s** | |
|---|---|---|---|
| **Publication Number** | **Publication Date** | **Publication Number** | **Publication Date** |
| WO 2014/153042 A1 | 25 September 2014 | WO 2014153042 A1 | 25 Sep 2014 |
| | | CR 20150504 A | 04 Jan 2016 |
| | | PH 12015502101 A1 | 11 Jan 2016 |
| | | US 2014271950 A1 | 18 Sep 2014 |
| WO 2016/154635 A2 | 29 September 2016 | WO 2016154635 A2 | 29 Sep 2016 |
| | | AU 2016238205 A1 | 02 Nov 2017 |
| | | CA 2980779 A1 | 29 Sep 2016 |
| | | US 2017079278 A1 | 23 Mar 2017 |
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| | | US 9456611 B2 | 04 Oct 2016 |
| | | ZA 201209505 B | 26 Nov 2014 |

**End of Annex**

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.