USE OF AN AGRICULTURAL COMPOSITION COMPRISING A MIXTURE OF AT LEAST TWO PLANT EXTRACTS SELECTED FROM CAPSICUM EXTRACT, MARIGOLD EXTRACT AND GARLIC EXTRACT AS DISINFECTANT FOR REMOVING SOIL PATHOGENS AND/OR NEMATICIDE

Use of an agricultural composition which comprises a mixture of at least two extracts selected from: Capsicum extract, Marigold extract and garlic extract as disinfectant for removing soil pathogens and/or as nematicide.
Description

Technical Field

[0001] The present invention relates to a formulation which acts as ecological disinfectant and as nematicide against pathogens in agricultural soils. This document describes its composition based on plant extracts, the production method and the application mode in soil infected by pathogenic microorganisms and/or nematodes.

Background Art

[0002] Infections in agricultural soils are a very important problem for farmers because it limits and reduces the production, while decreasing the quality of crops. In order to minimize infections in the soil, chemically synthesized disinfectants have been typically used, but usually they generate waste that remain in the soil for years and they can move to the crops. To this fact it must be added the important toxicological risks due to its handling. Alternatively, many plant extracts have been studied as a viable option to replace synthetic disinfectants, as these natural extracts have low or no toxicity and low environmental persistence. Secondary metabolites with antimicrobial activity which may be present in the plant extracts, belong mainly to the families of terpenoids, phenolic compounds, phenylpropanoids, stilbenes, alkaloids and saponins. These secondary metabolites have the advantage of being rapidly degraded in the soil, usually do not have a toxic effect on mammals and they can be used in organic farming systems.

[0003] Among these extracts, compound obtained from plants of the genus Capsicum have been studied, and they have been shown to exhibit antifungal, antimicrobial and slightly nematicidal effects (Cer: n-Carrillo et al, 2014; Moreno-Lemon et al, 2012; Santos-Neves et al., 2009). Analyses of these extracts indicated that the most important components thereof belong to the family of the capsaicinoids, like capsaicin, capsaicidine, capsidol, the capsinosides and capsicodendrin. These compounds present antibacterial and fungicidal properties, among other effects.

[0004] Antimicrobial action mechanisms of the capsaicinoids begin causing osmotic stress and damage to the structure of the plasma membrane. Of all the compounds found in extracts of plants of the genus Capsicum, the most important is capsaicin, which is an amide obtained from vanillylamide. Capsaicin establishes poorly specific interaction with lipids, aligning its alkyl chain with the acyl chains of the phospholipids, while the hydroxyl groups and the amide of the capsaicin are oriented towards the water surface. This location on the surface of the phospholipid membrane allows that the polar part of capsaicin can form hydrogen bonds with the polar head of phospholipid, thus affecting the packaging of lipids and modifying their thermotropic properties.

[0005] Despite the proven anti-fungal and slightly nematicides properties of the Capsicum extract, its action is not sufficient to disinfect agricultural soils with a medium and high infection level, so it should be combined with other plant extracts that present synergy effects with the aim of potentiate their action.

[0006] Marigold extract can be used as a nematicide, but also it is an important natural source of carotenoids, natural fat-soluble pigments synthesized by plants, algae and photosynthetic bacteria (E modi, 1978). Chemically, carotenoids are liposoluble tetraterpenes originated from the condensation of isoprenyl units, which give rise to a series of conjugated double bonds forming a chromophoric system. Due to its unsaturation, these compounds are sensitive to oxygen, metals, acids, peroxides, heat, light and lipooxygenase (Bego’a et al., 2001). Carotenoids have important antioxidant properties that have been demonstrated in numerous studies (Garrancoj auregui et al, 2011; Stahli and S ies, 2003) and also, they protect plants against abiotic stress, as well as being involved in the photoprotection of them (Cazzonelli, 2011).

[0007] Garlic extract (Allium sativum L.) is widely used as a repellent and insecticide, but it also has fungicidal properties (S ealy et al., 2007) and nematicide effects (Gupta and Sharma, 1991; Nath et al, 1982; Stop and Guzman, 1997). These activities are possible because garlic extract contains significant amounts of sulphurous bioactive substances with antifungal, antibacterial and nematicides properties like allicin, and other allyl sulfides like diallyl disulfide or diallyl trisulfide, among others; and dithiins like 3-vinyl-[4H]-1,2-dithiin or 2-vinyl-[4H]-1,3-dithiin. In addition, garlic extract is composed of substances of the families of phenols, flavonoids and proanthocyanidins, all of them with important antioxidant activity (Otunola and Afolayan, 2013, Rady and Seif El-Yazal, 2014). These antioxidants reduce levels of free radicals and inhibit their subsequent generation, which prevent cell aging. In plant organisms, all these antioxidants help plants to develop their own natural defence, making them stronger against external attacks. Due to its chemical composition, garlic extract could reinforce the action of the Capsicum extract in the formulation of a soil disinfectant.

[0008] Although the fungicidal and/or nematicide properties of these two extracts separately are known and they are being individually implemented in some agricultural formulations, the present invention relates to a mixture thereof to potentiate separately the effect of the extracts, taking advantage of the bioactive compounds of each extract that would be interesting in each case. It has been found that these extracts show synergy effects between them, so a mixture thereof potentiates the action that would have individually applied to low application rate per hectare.

[0009] With the mixture, a formulation with strong antifungal and antimicrobial effect by the presence of capsainoids from Capsicum would be achieved. Furthermore, a nematicide effect would be obtained us-
ing Marigold extract, which provides parallel antioxidant capacity of carotenoids and a protection effect of plants against abiotic stress. Finally, the nematicide and fungicide effect is reinforced by the bioactive sulphur substances from garlic extract, which also would be contributing phenolic compounds with antioxidant activity.

Summary of invention

[0010] Formulation object of this invention would be a mixture of plant extracts (Capsicum extract, Marigold extract and/or garlic extract), which acts against pathogens and/or nematicide as an ecological disinfectant for agricultural soils.

[0011] Knowing the action mechanisms of all plant extracts mentioned so far, the formulation of a soil disinfectant and nematicide as a mixture of at least two of said extracts is proposed.

- Capsicum Extract, at a maximum concentration of 95% by weight of the total.
- Marigold extract, at a maximum concentration of 95% by weight of the total.
- Garlic extract, at a maximum concentration of 95% by weight of the total.

[0012] The percentage in which each extract is added to the final formulation will depend on the effect that we want to achieve, thereby enhancing the properties of the extract (or extracts of interest in each case).

[0013] The physical mixture of the components of the formulation is carried out, and it is completed with the appropriate technological coadjuvants with the aim that the formulation has the best features for managing and it complies with the proposed objective.

[0014] The final product is an organic formulation with an important fungicide and nematicide effect, used as a disinfectant for agricultural soil. It is not selective nor as fungicide even as nematicide, so it acts against some types of pathogenic fungus and against any species of nematodes. It also has some ability to prevent germination of weeds, resulting in a fungicidal, bactericide, nematicide and pre-emergence herbicide effect for agricultural soils.

[0015] This formulation provides new technical advances over traditional disinfectants (DD, metam sodium, metam potassium, etc.), that allow to reach the same efficiency level and it solves problems in agricultural soils using more sustainable solutions.

[0016] After numerous agronomic researches a bout the functioning of disinfectant and nematicide object of the present invention, it has been found that the most important aspect to get the best results is the application mode and the soil management throughout the treatment process. How to apply the formulation depends on the desired objective. Due to agronomic studies carried out, two operation modes have been defined: function as a disinfectant for killing pathogens, and function as nematicide.

[0017] All this in accordance with the claims accompanying the present descriptive report and are incorporated herein by reference. Preferred embodiments of the invention are indicated in the dependent claims which also are incorporated herein by reference thereto.

[0018] Throughout the description and claims the word "comprise" and its variations are not intended to exclude other technical features, additives, components or steps. For those skilled in the art, other objects, advantages and features of the invention will arise partly from the description and partly from practice of the invention. The following examples and drawings are provided by way of illustration and are not intended to restrict the present invention. Furthermore, the present invention covers all possible combinations of preferred embodiments set forth herein.

Brief description of drawings

[0019] Below goes on to describe very briefly a series of drawings which aid in better understanding the invention and which are expressly related to an embodiment of said invention presented as a non-limiting example thereof.

Figure 1. Nematodes population level (%) for 14 days after application of the disinfectant and nematicide formulation.

Figure 2. Garlic production (%) depending on its size, in the control area and the area treated with disinfectant and nematicide formulation.

Description of embodiments

[0020] Use of extracts as disinfectants for removing soil pathogens.

[0021] In this case, the application dose of the preferential formulation was between 10 and 30 litres per hectare, according the required needs by the infection level of the soil and the soil characteristics where it will be applied.

[0022] The application mode of the disinfectant object of the present invention must follow these guidelines. First, a tillage of the soil must be done to achieve adequate aeration and proper soil texture, with the aim to facilitate a subsequently reactivity and diffusion of the active compounds formed. The action mode of the active substances contained in the formulation is primarily by contact, so it is necessary to facilitate maximum contact between disinfectant and pathogenic microorganisms.

[0023] Then, the soil should be irrigated to achieve a level of moisture that reaches the maximum field capacity, as water will be the carrier for the disinfectant diffusion. The effectiveness will be conditioned by the ability of the soil to keep the applied formulation if possible and to take place the contact between substances applied and pathogens. In this sense, it is necessary to consider
the soil structure for proper irrigation.

[0024] In very compacted soils, irrigation time should be increased to achieve correct water penetration and reach the proper humidity level and at the proper depth. In sandy soils that have no retention capacity it is also necessary to increase the irrigation time to reach the desirable capacity field without causing the disinfectant washing. Also, the holding capacity of these soils may be improved with appropriate products, as a contribution of organic matter through the irrigation system, either in solid or liquid composition.

[0025] Preferably, formulation must be applied through irrigation, spraying or micro-sprinkler irrigation. Devices that allow droplet sizes as small as possible and maximum coverage must be used, so that the greater diffusion must be obtained.

[0026] Once the disinfectant object of the present invention has been applied to the soil surface, it is preferable to perform a new irrigation to enhance the penetration and diffusion of the product into the soil.

[0027] It is necessary to wait a period of at least 10 days before planting any crop with the aim that the fungicidal effect is completed. Disinfection with this formulation can be performed prior to implantation of any type of crop.

[0028] Fungicidal action of the formulation object of the present invention acts against any pathogenic soil fungus and this fact is achieved in three stages:

- A first stage of organic oxidation, which occurs once the product has been incorporated and distributed over the soil. During this stage, organic reactions occur with the aim to oxidize the organic fraction of soil and the pathogens. In this case it is very important that the soil texture is adequate, so it is recommended to carry out the tillage and irrigation procedure described above. Tillage allows the formulation penetrates in all infected zones, while irrigation water acts as diffusion carrier.

- A second stage of allelopathic contact, in which a total destruction of the cell walls of biotic organisms occurs. These cell walls were already partly denatured by means of prior oxidation reactions. As a result, the final destruction of membranes is obtained by inhibiting the enzymes responsible for making the substances contained therein.

- The last stage is a microbial regeneration, caused by elimination of pathogens microorganisms and contaminants from soil. This fact improves the physicochemical properties of soil, with a decrease in conductivity, pH and content of oxidized humus, which then allows the activation of beneficial microbial flora native which had been displaced by pathogens. This practice represents an important improvement of the physicochemical and microbiological features of the soil cultivation and its quality level, allowing greater viability of crops.

[0029] The use of this formulation as pathogens disinfectant in agricultural soils has significant advantages over conventional disinfectants which are currently used in agriculture. First, to achieve a similar control level, the dose of the formulation object of this invention is much lower than common disinfectants, which lower cost and lower consumption of resources is obtained. In addition, conventional products have significant toxicological and environmental risks, so much so that it must be applied by trained personnel. However, the disinfectant formulation described here is a product that poses no risk of significant toxicity or environmental pollution, obtaining a much more sustainable disinfection of soil.

Test 1

[0030] In order to illustrate the disinfecting action of the present invention, the following test describes the application of the formulation on a greenhouse infected by various types of pathogens.

[0031] The application of the formulation was performed in half of a parcel subsequently planted with courgettes and following the application procedure described herein at a dose of 10 L/ha, keeping the other half of the parcel as a control area.

[0032] The effectiveness of the formulation was measured as a function of the obtained production (kg/ha), knowing that the production decreases when soil infection increases. Considering the type of crop used for the test (courgettes), production was continued for three consecutive weeks in the two halves of the parcel (infected control area and disinfected area). Results obtained about production are shown in Table 1, where it can be noted that production increased in the area treated with disinfectant from the first week compared to the control area, resulting an increase of 25% of production after three weeks of study. According to previous assumptions, it can be concluded that the application of the formulation object of the present invention decreased the level of soil infection and consequently increased the production.

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[0033] Table 1. Courgette production (kg/ha) in greenhouses, on a soil infected by fungi, and untreated (control) and after treatment with the formulation (disinfectant).
Nematicide effect.

[0034] This formulation can be applied with any type of soil cultivation. The preferred application dose was between 2.5 and 10.0 litres of formulation per hectare. A previous and proper soil management is needed to ensure that the nematicide comes into contact with nematodes to eliminate. To do this, previously to the application of the formulation, a bundant irrigations must be performed with the aim to reach the maximum field capacity or at least reach 70% of the field capacity. It is also necessary to note that the irrigation depends on the soil structure. Therefore, in compacted soils, irrigation must be more abundant because water must penetrate properly. Similarly, for sandy soils the irrigation also must be abundant to reach the desired field capacity.

[0035] Preferably, the application of the formulation would be done by irrigation systems (pivot, droppers, sprays or nebulizers), but also the product can be applied via foliar application with treatments directed to the ground. The most efficient systems are those which allow greater and better dissemination, such as those with smaller nozzles and higher coverage on the field. In sandy soils, where the product can be washed from strong irrigations, the formulation should preferably be applied together with organic matter, amino acids or both simultaneously, with the aim to increase their fixation and stability in soil.

[0036] Preferably, after application of the formulation, it is necessary to irrigate the soil to promote maximum dispersion and maximum contact of the active substances of the formulation with nematodes.

[0037] The nematicidal effect of the formulation object of the present invention acts against adults and larvae of any species of nematode, and it occurs by contact and ingestion, presenting also penetration in the first vegetative layers of the root.

[0038] When the contact and/or ingestion of the formulation by nematodes (larvae and/or adults) occurs, the membrane and the cysts or nodules coating are destroyed, inhibiting the enzymes responsible to produce substances containing the nematode cuticle body. Consequently, nematode immobilization is produced and the death of 50% of the population in less than 24 hours and 80-100% in less than 96 hours from the application occurs.

[0039] This formulation also has systemic power up to radicular rhizodermis zone, where it contacts with nematode larvae or adults found therein. Due to this capacity to penetrate, phenomena of rhizogenesis activation in the root system are activated by the proper effect of the formulated at the root.

[0040] Preferably, the application of the formulation on the soil infected with nematodes must be performed at the following times:

- At the beginning of the crop cycle and after germination in soils with nematodes. The application currently decreases the level of adults, which are dormant when weather conditions are not suitable for their activation. This allows achieving a reduction in nematode adult population level but not the level of eggs present in the cultivation soil.
- When the activation of the nematode begins and the population level by eggs hatch present in the soil goes up, coinciding with the start of root crop activity (elevated temperature and soil moisture). This second application of the formulation is of great importance to control the new larvae hatching from eggs existing in soil before passing to adulthood and carry put new eggs, thus in this phase a decreased level of eggs respects the initial. If the larvae are not controlled at this time, its development would entail a further increase of adults and new clutches of eggs, exponentially increasing the population levels in a few days.
- After sufficient time (10-25 days), all eggs that may have still stay on the floor with prior treatment begin to hatch, starting the cycle larval development again. In this case, a third application of the formulation is recommended to eliminate all the new larvae before they reach adulthood. A new egg laying is avoided and, a control between 75 and 100% of the starting population level and a very significant reduction in the presence of eggs in the soil is achieved. As a result in greater production and high fruit quality is obtained, and a minimum level of infection soil for future crops.

[0041] Novelties and advantages of the formulation object of this invention against commercial nematicides are:

- First, a higher level of control population of nematodes in soil, reaching percentages 75-100% in the case of this formulation against much lower levels with commercial products.
- In addition, control of nematodes is achieved reducing the negative effects on crops, resulting in increased production and better quality, especially regarding the size and specific weight of the fruits.
- Finally, toxicological and environmental exposures to chemicals are significantly decreased, as this is an ecological formulation without term security for crops due to its almost instantaneous biodegradation, also decreasing the cost of the overall for the cultivation.

Test 2

[0042] For checking the power of the nematicide formulation described in this invention, an application test of the formulation over a garlic cultivation infected by nematodes has been done.

[0043] Inside the plantation, two different areas were differentiated, leaving one of them as untreated control, and over the other one the proposed formulation was...
applied at a dose of 5 L/ha through irrigation.

[0044] Population level of adult nematodes, young larvae and larvae previously infective treatment was measured. Results after 3, 7 and 14 days are shown in Figure 1.

[0045] The application of the disinfectant and nematocide formulation reduced quickly the nematode population level during the first week, especially in the case of young larvae. After two weeks the level of nematodes was between 15 and 20% of the initial.

[0046] Considering that the infection level of the soil affects both production yield and quality of the crop, after harvesting both areas was performed, the garlic size was measured according the classification established for garlic depending on their size. Low to larger sizes were differentiated: second, first, flower, super, extra, jumbo, super jumbo and elephant.

[0047] Figure 2 shows the percentage of production found for each garlic size. It can be seen that in the control area without treatment, size was lower than corresponding to the area treated with disinfectant and nematicide object of the present invention.

[0048] This result indicates a decrease of nematode population level during harvest. This fact is due to the application of this product that improves the overall size of garlic because the crop can develop according to their normal cycle.

Citation list

[0049]


Claims

1. Use of an agricultural composition containing a mixture of at least two plant extracts as disinfectant for removing soil pathogens and/or nematicide, being these extracts selected from: Capsicum extract, Marigold extract and garlic extract.

2. Use of a composition according to claim 1 which comprises Capsicum extract in a maximum weight percentage of 95%.

3. Use of a composition according to claim 1 which comprises Marigold extract in a maximum weight percentage of 95%.

4. Use of a composition according to claim 1 which comprises garlic extract in a maximum weight percentage of 95%.

5. Procedure for pathogen removal from agricultural soil which comprises the steps of: (i) to irrigate agricultural soil; (ii) use of an agricultural composition according to any of claims 1 to 4 by watering, spraying or micro-spraying; (iii) to irrigate agricultural soil again; and (iv) to wait ten days
prior to planting the selected crop.

6. Procedure for nematodes removal from agricultural soil which comprises the steps of: (i) to irrigate agricultural soil; (ii) use of an agricultural composition according to any of claims 1 to 4 by watering or foliar application; (iii) to irrigate agricultural soil again; and to repeat steps i-iii: (a) at the beginning of the crop cycle and after germination in soils with presence of nematodes; (b) when nematode activation begins and the population level by eggs hatch present in the soil rises, coinciding with the beginning of root activity of the culture; and (c) after 10 and 25 days between the second application of step b).
### INTERNATIONAL SEARCH REPORT

**EP 3 357 340 A1**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A01N65/12  A01N65/38  A01N65/42  A01P1/00  A01P3/00  A01P5/00

**ADD.**

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A01N

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

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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**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

"S" document member of the same patent family

**Date of the actual completion of the international search** 24 November 2016

**Date of mailing of the international search report** 27/01/2017

**Name and mailing address of the ISA/ Authorized officer**

European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk
Tel. (+31-70) 340-0040, Fax. (+31-70) 340-3016

Lorenzo Varela, M
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INTERNATIONAL SEARCH REPORT

Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. □ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. □ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying an additional fee, the Authority did not invite payment of additional fees.

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims, it is covered by claims Nos.:

1-6(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-6(partially)

1) the use of an agricultural composition comprising a mixture of at least two plant extracts being Capsicum extract and Marigold extract as disinfectant for removing soil pathogens and/or nematicide, and 2) a procedure for pathogen removal from agricultural soil which comprises the use of the above-mentioned composition.

2. claims: 1-6(partially)

1) the use of an agricultural composition comprising a mixture of at least two plant extracts being Capsicum extract and garlic extract as disinfectant for removing soil pathogens and/or nematicide, and 2) a procedure for pathogen removal from agricultural soil which comprises the use of the above-mentioned composition.

3. claims: 1-6(partially)

1) the use of an agricultural composition comprising a mixture of at least two plant extracts being Marigold extract and garlic extract as disinfectant for removing soil pathogens and/or nematicide, and 2) a procedure for pathogen removal from agricultural soil which comprises the use of the above-mentioned composition.
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REFERENCES CITED IN THE DESCRIPTION

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Non-patent literature cited in the description