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PATENTS ACT, 1978

CERTIFICATE

accordance with section 44 (1) of the Patents Act, No. 57 of 1978, it is hereby certified that:

Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences

Has been granted a patent in respect of an invention described and claimed in complete specification deposited at the Patent Office under the number

2022/13199

opy of the complete specification is annexed, together with the relevant Form P2.

In test hereof, the seal of the Patent Office has been affixed at Pretoria with effect from the 26th day of April 2023

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54	Title of invention
APPLICATION OF NATURAL MONOTERPENE COMPOUND SABINENE IN PREPARATION OF HERBICIDE	

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TITLE OF INVENTION

54	APPLICATION OF NATURAL MONOTERPENE COMPOUND SABINENE IN PREPARATION OF HERBICIDE

APPLICATION OF NATURAL MONOTERPENE COMPOUND *SABINENE* IN PREPARATION OF HERBICIDE

TECHNICAL FIELD

The invention relates to the technical field of applying natural compounds in plant volatile oil to herbicides, and in particular to an application of a plant volatile oil component *sabinene* in herbicides.

BACKGROUND

Chemical herbicides are widely used, but many synthetic chemical herbicides are easy to remain in nature for a long time due to the lack of corresponding microorganisms to decompose them, thus threatening the safety of soil and groundwater resources what human beings depend. As herbicides, the natural ingredients extracted from nature are paid more and more attention because of their easy decomposition and no residue in nature. In addition, although some chemical herbicides are decomposed in the soil, their targets are single. As a result of long-term and large-scale application, weeds gradually evolve resistance, and the efficacy of the herbicides is reduced or even lost. In this case, it is particularly important to develop herbicides with novel targets and mechanisms. The natural products with rich species, different configurations and diverse action mechanisms in nature provide the possibility for the production of new herbicides. The herbicidal activity of some natural products is comparable to that of chemical herbicides, such as 1,8-Elaeagnum, a natural volatile component. There are also a variety of natural herbicides whose efficacy may not be compared with that of chemically synthesized herbicides at the

beginning of the discovery, but their efficacy are greatly enhanced after the chemical structure transformation in the future, thus having the value of commercial popularization, such as sulcotrione transformed from phytotoxin cellophane in *Callistemon rigidus* R.Br.

Sabinene (Bicyclo [3.1.0] hexane, 4-methyl-1-(1-methylethyl)-) is a monoterpene compound, and is widely distributed in the volatile oils of many plants. *Sabinene* has been reported to have the activities of killing pests, diminishing inflammation and inhibiting microbial growth, etc., referring to references 1-5; but the growth inhibition (phytotoxicity) of *Sabinene* on other plants has not been reported at home and abroad. At present, there are reports about its biosynthesis methods, referring to reference 6.

Sabinene is an important natural monoterpene compound, and it is used as a condiment, perfume additive, fine chemical and advanced biofuel. However, the objective of using it as a herbicide has not been reported. Microbial synthesis is an effective way to prepare *sabinene*. At present, the preparation methods are mature, but there is no mass production yet.

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2. Wang, Y., You, C.X., Yang, K., et al. Bioactivity of essential oil of Zingiber purpureum rhizomes and its main compounds against two stored product insects. Biological and Microbial Control, 2015, 108(3), 925-932.

3. Matias, E.F.F., Alves, E.F., Silva, M.K.N., et al. Seasonal variation, chemical composition and biological activity of the essential oil of Cordia verbenacea DC (boraginaceae) and the *sabinene*. Industrial Crops & Products, 2016, 87, 45-53.

4. Benelli, G., Flamini, G., Canale, A., etal. Repellence of Hyptis suaveolensl. (Lamiaceae) whole essential oil and major constituents against adults of the granary weevil sitophilus granaries (l.) (coleoptera: dryophthoridae). Bulletin of Insectology, 1967, 31 (65), 177-183.

5. Wang, C.F., Yang, K., Zhang, H.M., et al. Components and insecticidal activity against the maize weevils of *Zanthoxylum schinifolium* fruits and leaves. Molecules, 2011, 16(4), 3077-3088.

6. Cao, Y., Zhang, H., Liu, H., et al. Biosynthesis and production of *sabinene*: current state and perspectives. Applied Microbiology and Biotechnology, 2018, 102:1535–1544.

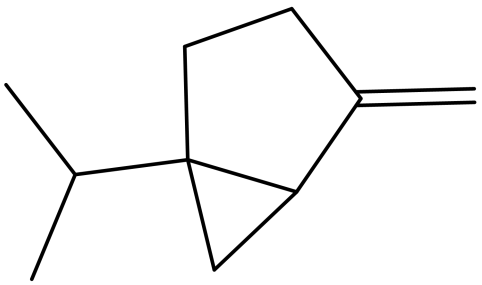
SUMMARY

The objective of the present invention is to provide an application of a natural monoterpene compound *sabinene* in a preparation of herbicides. The research test shows that the compound kills monocotyledonous and dicotyledonous plants, *Setaria viridis,* and has obvious inhibitory effect on dicotyledonous plants, *Amaranthus retroflexus* and *Medicago sativa* when the compound is applied at a low concentration of 5 μl/mL. This compound is a component of plant volatile oil, with fragrant smell. It not only has herbicidal activity, but also has a certain insect repelling effect. The compound is naturally degraded and has no pollution to the environment when it is prepared into different concentrations and used alone or in combination with other pesticides before or at seedling stage. It is used as an herbicide, and has great potential commercial

value.

An application of a natural monoterpene compound *sabinene* in a preparation of herbicides of the invention.

The application of a natural monoterpene compound *sabinene* in a preparation of herbicides of the invention, wherein a structural formula of the compound is:



The application of a natural monoterpene compound *sabinene* in a preparation of herbicides of the invention, wherein the compound is widely existing in plant volatile oil, and research experiments show that it has a significant effect on the growth and yield of seedlings of common weeds *Setaria viridis*, *Medicago sativa* and *Amaranthus retroflexus* at a low concentration of 2 μl/mL. This growth inhibition gradually strengthens with an increase of concentration. When the concentration reaches 2 μl/mL, the inhibition rates of *sabinene* on the root length of *Setaria viridis*, *Medicago sativa* and *Amaranthus retroflexus* are 71%, 67% and 89% respectively. When the concentration increases to 5 μl/mL, the monocotyledonous plants *Setaria viridis* seeds are basically killed, the inhibition rates of this compound on the root length of *Medicago sativa* and *Amaranthus retroflexus* reach 78% and 84%, respectively, and the seedlings lost an ability to continue growing. The natural compound belongs to monoterpene, and its biosynthesis has been reported. The herbicidal activity of the compound has not been reported at home and abroad.

The application of a natural monoterpene compound *sabinene* in a preparation of herbicides of the invention, wherein the compound is obtained from widely distributed plant volatile oil, such

as *Dracocephalum integrifolium* volatile oil, by solvent extraction and thin layer chromatography, or the compound is synthesized by biological fermentation. This compound naturally exists in the volatile oils of many plants and is decomposed in nature, so it does not cause any pollution to the environment.

The application of a natural monoterpene compound *sabinene* in a preparation of herbicides of the invention, wherein the compound is prepared into different concentrations, used alone or mixed with other pesticides before or at seedling stage, dissolved in distilled water containing 0.5% dimethyl sulfoxide (DMSO) or acetone by volume, and sprayed before and after the emergence of weeds according to a spraying concentration of 10-100 μl/mL to be used as herbicides.

In addition, the compound is also used as a precursor to modify and transform its chemical structure, and then be commercialized after the herbicidal activity of the compound is further improved.

The application of a natural monoterpene compound *sabinene* in a preparation of herbicides of the invention has the following beneficial effects.

Firstly, the *sabinene* provided by the invention obviously inhibits the seedling growth of three common weeds, namely *Setaria viridis, Medicago sativa* and *Amaranthus retroflexus*, at a low concentration of 2 μl/mL. This growth inhibition gradually strengthens with the increase of concentration. When the concentration reached 2 μl/mL, the inhibition rates of *sabinene* on the root length of *Setaria viridis, Medicago sativa* and *Amaranthus retroflexus* are 71%, 67% and 89% respectively. When the concentration increases to 5 μl/mL, the monocotyledonous plant *Setaria viridis* seeds are basically killed, the inhibition rates of the compound on the root length of

Medicago sativa and *Amaranthus retroflexus* reach 78% and 84% respectively, and the roots of the seedlings are obviously distorted and turned dark brown, showing that the seedlings have been seriously poisoned. At this concentration, the seedlings stop growing and die gradually. At 5 μl/mL, the inhibition rates of *sabinene* on the seedling height of *Medicago sativa* and *Amaranthus retroflexus* all reach 83%, but the inhibition rate of *Setaria viridis* still reaches 100%. The results show that the compound has a stronger effect on monocotyledonous plant *Setaria viridis* than dicotyledonous plants *Amaranthus retroflexus* and *Medicago sativa*.

Secondly, the compound *sabinene* is suitable for weed control in farmland. The compound is a natural component, and is degraded naturally, so it is harmless to people and animals, and has a fragrant smell. After application, the compound does not cause pollution to the air, and it has a certain insect repellent effect. The compound is applied to agricultural production, especially to weed control in organic agriculture. The compound has strong herbicidal activity against three common weeds, namely *Setaria viridis, Medicago sativa* and *Amaranthus retroflexus*, and is applied to control them and other weeds in farmland. The herbicide of the invention is natural, non-toxic and harmless, and may be decomposed in nature, so it is environment-friendly and does not pollute the environment.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows an inhibitory effect of *sabinene* on the root length of *Setaria viridis, Medicago sativa* and *Amaranthus retroflexus* seedlings, with concentrations of 0.25 μl/ml, 0.5 μl/ml, 1 μl/ml, 2 μl/ml and 5 μl/ml respectively.

FIG. 2 shows an inhibitory effect of *sabinene* on the seedling height of *Setaria viridis, Medicago sativa* and *Amaranthus retroflexus*, with concentrations of 0.25 μl/ml, 0.5

μl/ml, 1 μl/ml, 2 μl/ml and 5 μl/ml respectively.

FIG. 3 is a graph showing the influence of *sabinene* on the growth of *Setaria viridis, Amaranthus retroflexus* and *Medicago sativa*.

DESCRIPTION OF THE INVENTION

The following embodiments will further illustrate the present invention, but the present invention is not limited to the following embodiments.

All raw and auxiliary materials, reagents and instruments selected in the invention are well known in the art, and other reagents and equipment well known in the art are applied to the implementation of the following embodiments of the invention.

Embodiment 1

Effects of *sabinene* on the growth of common weeds seedlings, taking *Setaria viridis, Medicago sativa* and *Amaranthus retroflexus* as examples;

preparing *sabinene* with a purity of 95% and distilled water with volume fraction of 0.5% acetone into 10 μ L/mL of emulsion suspension, and then diluting the prepared emulsion suspension to 0.25μl/mL，0.5 μl/mL，1 μl/mL，2 μl/mL， and 5 μl/mL successively with distilled water containing 0.5% acetone by volume; placing filter paper in a culture medium with a diameter of 9 cm, and then adding 4mL of 0.25μl/mL，0.5 μl/mL，1 μl/mL，2 μl/mL， and 5 μl/mL of the prepared emulsion suspension; adding 10 seeds of *Setaria viridis, Medicago sativa* and *Amaranthus retroflexus* in each culture medium, treating each medium for three times, sealing them with sealing film, then putting them into a 25℃ incubator to culture for 5 days, and

measuring the seedling height and root length of the seedlings.

Statistical methods: first, the single factor variance is used to test whether the differences among the data of each group are significant, and then LSD method is used to analyze the data. The differences among the groups with different marker letters are significant, and the level is > 0.05.

Results: at a low concentration of 2 μl/mL, the growth of *Setaria viridis, Medicago sativa* and *Amaranthus retroflexus* seedlings is significantly inhibited, and when the concentration increases, the affected degree gradually increases. At a low concentration of 0.5 μl/mL, the compound begins to have a significant inhibitory effect on *Medicago sativa* and *Setaria viridis,* with root length inhibition rates of 35% and 32% respectively, and seedling height inhibition rates of 28% and 20% respectively. The effect of the compound gradually increases with the increase of concentration. The compound has no such effect on *Amaranthus retroflexus* at this concentration, until the concentration reaches 2 μl/mL, and *Amaranthus retroflexus* begins to show the same significant inhibitory effect as the other two tested plants. At the concentration of 2 μl/mL, the root lengths of *Medicago sativa*, *Amaranthus retroflexus* and *Setaria viridis* are 29%, 33% and 11% of the control, and the seedling heights are 26%, 18% and 12% of the control, respectively. When the concentration increases to 5 μl/mL, the monocotyledonous plant *Setaria viridis* seeds are basically killed, the root lengths of *Medicago sativa* and *Amaranthus retroflexus* are only 22% and 16% of that of the control, and the roots of seedlings are obviously distorted and the root tips are rotted, showing that the seedlings have been seriously poisoned. At 5 μl/mL concentration,

the inhibition rates of the compounds on the seedling heights of *Medicago sativa* and *Amaranthus retroflexus* are 83% and 83% respectively. At high concentration, the compound has stronger effect on monocotyledonous plant *Setaria viridis* seeds than two dicotyledonous plants, showing that the killing effect of the compound on monocotyledonous plants may be stronger than that on dicotyledonous plants; referring to FIG 1 and FIG 2 for specific effects.

Embodiment 2

Effects of *sabinene* on the growth of common weeds seedlings, taking *Setaria viridis, Medicago sativa* and *Amaranthus retroflexus* as examples;

preparing *sabinene* with a purity of 95% and distilled water with volume fraction of 0.5% acetone into 100 μ L/mL of emulsion suspension, uniformly spraying the emulsion suspension on the healthy potted plants of *Setaria viridis, Medicago sativa* and *Amaranthus retroflexus* seedlings after the emulsion suspension is shaken evenly, and observing the killing effect on the plants after 12 h overnight.

Results: after 12 h overnight, the seedlings of *Setaria viridis, Medicago sativa* and *Amaranthus retroflexus* show obvious albinism and wilt, achieving the effect of herbicide, and referring to FIG. 3 for specific effect.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS

1. An application of a natural monoterpene compound *sabinene* in a preparation of herbicides.

FIGURES

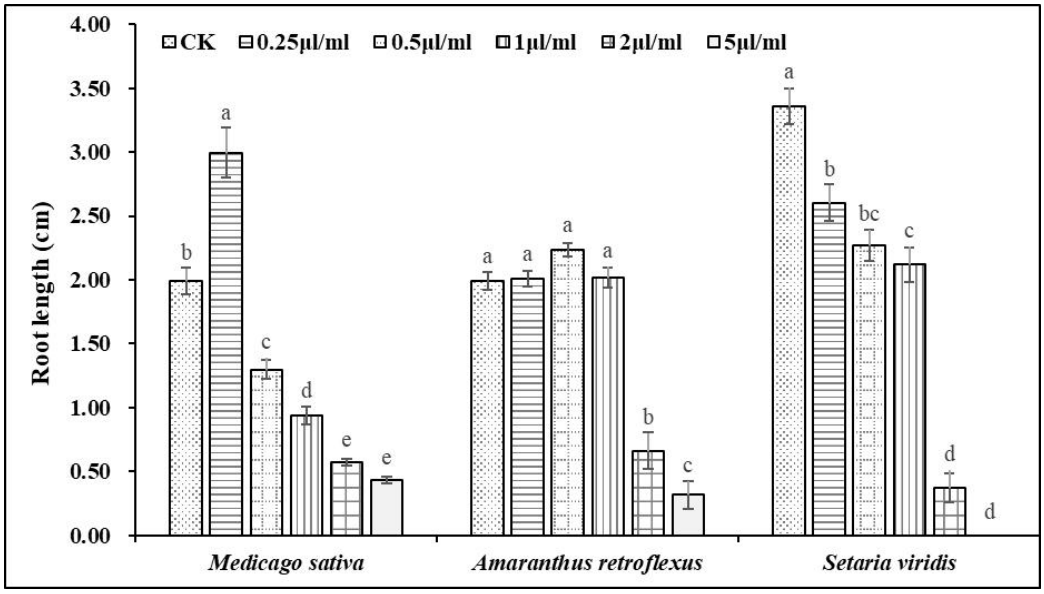


FIG. 1

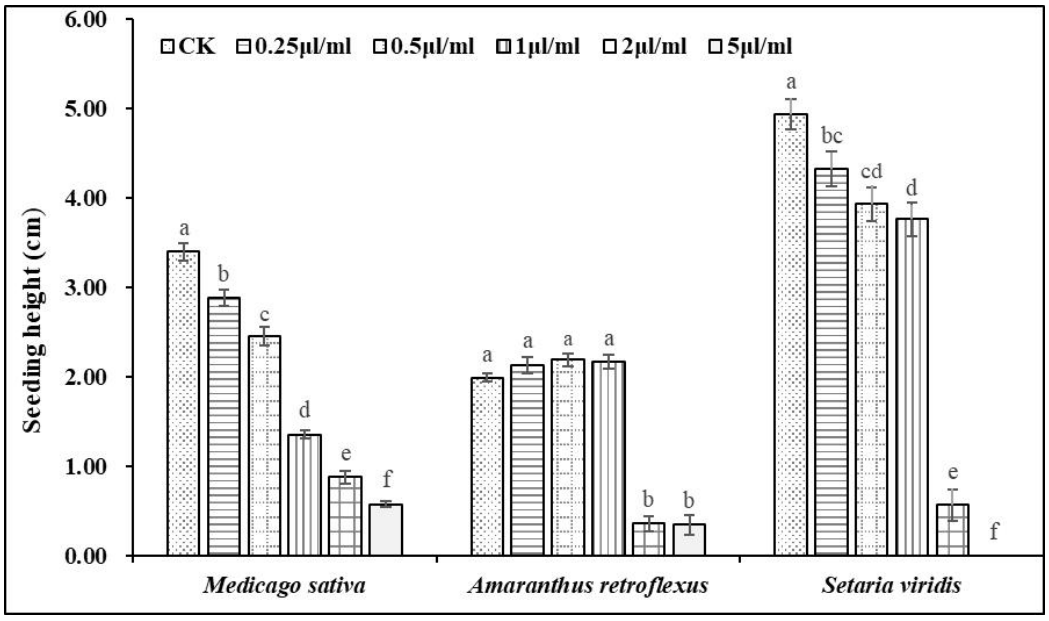


FIG. 2

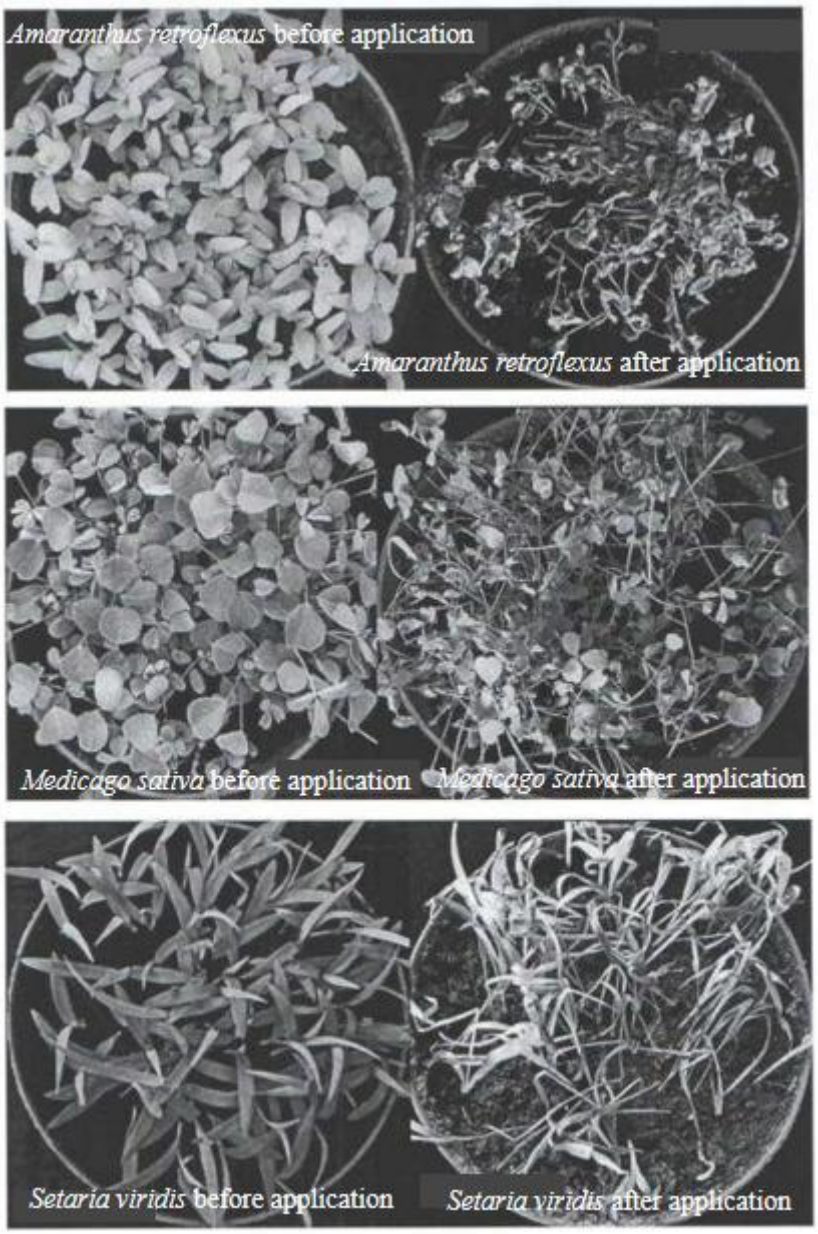

Amaranthus retroflexus before application
Amaranthus retroflexus after application
Medicago sativa before application
Medicago sativa after application
Setaria viridis before application
Setaria viridis after application

FIG. 3

ABSTRACT

This invention relates to an application of a natural monoterpene compound *sabinene* in a preparation of herbicides. The research test shows that the compound kills monocotyledonous and dicotyledonous plants, *Setaria viridis,* and has obvious inhibitory effect on dicotyledonous plants, *Amaranthus retroflexus* and *Medicago sativa* when the compound is applied at a low concentration of 5 microliter per milliliter. This compound is a component of plant volatile oil, with fragrant smell. It not only has herbicidal activity, but also has a certain insect repelling effect. The compound is naturally degraded and has no pollution to the environment when it is prepared into different concentrations and used alone or in combination with other pesticides before or at seedling stage. It is used as an herbicide, and has great potential commercial value.