

COMMONWEALTH of AUSTRALIA

PATENTS ACT 1952

APPLICATION FOR A STANDARD PATENT

609 797

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We

KIYOSHI SOTOME
of 4-10, Setagaya 2-chome, Setagaya-ku,
Tokyo, Japan

LODGED AT SUB-OFFICE

11 APR 1988

Melbourne

hereby apply for the grant of a Standard Patent for an invention entitled:

"A METHOD OF PROTECTING CROPS BY A NON-TOXIC COMPOSITION"

which is described in the accompanying ~~provisional~~
complete specification.

Details of basic application(s):—

Number

Convention Country

Date

88950/1987

Japan

13th April, 1987

APPLICATION ACCEPTED AND AMENDMENTS

ALLOWED.....19.2.91.....

The address for service is care of DAVIES & COLLISON, Patent Attorneys, of 1 Little
Collins Street, Melbourne, in the State of Victoria, Commonwealth of Australia.

Dated this 11th day of April, 1988

To: THE COMMISSIONER OF PATENTS

H. M. Rimington

(a member of the firm of DAVIES &
COLLISON for and on behalf of the Applicant).

Davies & Collison, Melbourne and Canberra.

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952-1973

DECLARATION IN SUPPORT OF CONVENTION OR
NON-CONVENTION APPLICATION FOR A PATENT
OR PATENT OF ADDITION

Insert title of invention.

Insert full name(s) and address(es)
of declarant(s) being the appli-
cant(s) or person(s) authorized to
sign on behalf of an applicant
company.

In support of the Application made for a ~~patent~~
~~patent of addition~~ for an invention
entitled: A method of protecting crops by a non-toxic
composition
I ~~Wa~~ Kiyoshi Sotome
of 4-10, Setagaya 2-chome, Setagaya-ku,
Tokyo, Japan

Cross out whichever of paragraphs
1(a) or 1(b) does not apply

1(a) relates to application made
by individual(s)
1(b) relates to application made
by company; insert name of
applicant company.

do solemnly and sincerely declare as follows:-

1. (a) I am the applicant..... for the ~~patent~~
~~We are~~ ~~patent of addition~~
or (b) ~~I am authorized by:~~

Cross out whichever of paragraphs
2(a) or 2(b) does not apply

2(a) relates to application made
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tor(s).

~~the applicant..... for the patent~~
~~patent of addition~~ ~~to make this declaration on its behalf.~~

2. (a) I am the actual inventor..... of the invention
~~We are~~
or (b)

~~is the actual inventor..... of the invention and the facts upon which the applicant.....~~
~~is~~
~~are~~ ~~entitled to make the application are as follows:-~~

State manner in which applicant(s)
derive title from inventor(s)

Cross out paragraphs 3 and 4
for non-convention applications.
For convention applications,
insert basic country(s) followed
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3. The basic application..... as defined by Section 141 of the Act ~~was~~
~~was~~ made
in Japan on the April 13, 1987
by Kiyoshi Sotome
in on the
by
in on the
by

4. The basic application..... referred to in paragraph 3 of this Declaration ~~was~~
~~was~~ the first application..... made in a Convention country in respect of the invention the subject
of the application.

Insert place and date of signature.

Declared at Tokyo, Japan this 22nd day of March, 1988

Signature of declarant(s) (no
attestation required)

Note: Initial all alterations.

Kiyoshi Sotome
Kiyoshi Sotome

DAVIES & COLLISON, MELBOURNE and CANBERRA.

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(57) Claim

1. A method of protecting crops against attack by insect pests, microorganisms and pathogenic microbes, the method comprising the application to the crops of a composition comprising cinnamic aldehyde and an antioxidant.

COMMONWEALTH OF AUSTRALIA

PATENT ACT 1952

COMPLETE SPECIFICATION

(ORIGINAL)

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COMPLETE SPECIFICATION FOR THE INVENTION ENTITLED:

"A METHOD OF PROTECTING CROPS BY A NON-TOXIC COMPOSITION"

The following statement is a full description of this invention,
including the best method of performing it known to me :-

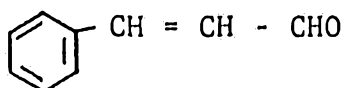
BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a method of protecting crops and more particularly, it is concerned with a method of protecting crops, whereby the protecting effect can be maintained in stable manner for a long time by the use of a non-toxic and stable composition containing cinnamic aldehyde as a predominant component.

2. Description of the Prior Art

Cinnamic aldehyde is a non-toxic material described in the official document as a food additive, which is represented by the following chemical structural formula:



The inventor has noted that cinnamic aldehyde has an excellent microbe controlling effect and can favorably be applied to protection of crops and has thus proposed a method of protecting crops characterized by spraying an emulsion of cinnamic aldehyde on the crops and thus protecting the crops from insect pests, microorganisms and pathogenic microbes, as disclosed in Japanese Patent Publication No. 25682/1986. In this method, an emulsion of cinnamic aldehyde, for example, emulsified with a nonionic surfactant consisting of a condensate of ethylene oxide and propylene oxide is sprayed on crops to protect from

molds such as ascomycetes, deuteromycetes, etc., pathogenic microbes such as phycomycetes, basidiomycetes, bacteria, etc. and insect pests such as cockroach carrying virus pathogenic microbes. This method is particularly effective for preventing economical loss due to damage by blight and noxious insects in the harvesting season for the health care of producers and for the preventive maintenance of soils.

Furthermore, the inventor has also proposed a method of protecting crops, characterized by applying a fertilizer with cinnamic aldehyde or a cinnamic aldehyde derivative to the soil, as disclosed in Japanese Patent Publication No. 32283/1986. This method is a novel and effective fertilizing method wherein the soil is fertilized with a fertilizer with cinnamic aldehyde or its derivative such as halogen derivatives thereof and thus subjected to disinfection in non-toxic manner while inhibiting harmful microbes without affecting useful bacteria, thereby maintaining a B/F value well-balanced.

Cinnamic aldehyde is a material which is non-toxic and has an excellent microbe controlling effect, but on the other hand, the stability of the microbe controlling effect is insufficient and the cause thereof has not been made clear, as described in Japanese Patent Application OPI (Kokai) No. 52236/1975 relating to a method of protecting foods with cinnamic aldehyde and a liquid bulb product. For the protection of crops, however, the stability of this effect for a long time has particularly been desired. Since cinnamic aldehyde has

widely been used as a spice for foods, but has not been used for the protection of crops until the inventor has proposed, study of the stability for a long period of time has not been made.

5

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method of protecting crops using non-toxic cinnamic aldehyde, whereby a stable protecting effect can be obtained for a long time.

It is another object of the present invention to provide a method of protecting safely and economically crops using a novel composition containing cinnamic aldehyde, which is non-toxic and has an effect of selectively controlling or inhibiting only harmful microbes in the soil and selectively inhibiting the normal performance of *Meloidogyne incognita*, the effect being stably maintained.

These objects can be attained by a method of protecting crops, which comprises applying or spraying a composition comprising cinnamic aldehyde and an antioxidant on crops and thereby protecting the crops from insect pests, microorganism and microbes.

DETAILED DESCRIPTION OF THE INVENTION

The inventor has made studies on improvement of the stability of the protecting effect of cinnamic aldehyde when

applied to crops and consequently, have found that the microbe inhibiting effect can be maintained by adding an antioxidant to cinnamic aldehyde to inhibit the oxidation of cinnamic aldehyde. The present invention is based on this finding.

5

Accordingly, the present invention provides a method of protecting crops against attack by pathogenic microbes and insect pests, which comprises applying or spraying a composition comprising cinnamic aldehyde and an antioxidant on crops and thereby protecting the crops therefrom.

10 In particularly preferred embodiments of the present invention, an emulsion comprising cinnamic aldehyde, an antioxidant and an emulsifier, or a composition comprising, on a support, cinnamic aldehyde and an antioxidant is used as the above described composition.

15 Various experiments relating to the protection of crops were carried out using the composition of the present invention, thus finding a surprising fact that the combination of cinnamic aldehyde and an antioxidant results in not only the above described antioxidant effect but also the following two large effects.

20 The first effect is a selective effect of inhibiting harmful microbes in the soil, i.e. pathogenic molds such as Fusarium, Pythium, Rhizoctonia, Phytophthora capsici, Pseudomonas and Corticium rolfsii without affecting useful microorganisms such as competitive yeasts, antinomycetes and useful molds.

The second effect is a breeding action of Steinernema

feltiae having no damage on plant roots in spite of inhibiting the normal performance of Meloidogyne incognita. The second effect is suggestive of development of a new protecting method of crops utilizing the antibiosis by microorganism instead of disinfection of the soil with harmful chemical materials by forwarding the study.

Protection of crops by the use of the composition comprising cinnamic aldehyde and an antioxidant according to the present invention can be carried out by any of methods of irrigating the soil or spraying on plants, in which the parts or positions of the plant to be applied and the application time are not limited and the plant can wholly be treated, as will in detail be illustrated in the following Examples.

The composition of the present invention comprises cinnamic aldehyde and an antioxidant. As the antioxidant, there can be used various materials having oxidation inhibiting action, for example, vitamin E, n-propyl gallate, BHT, eugenol and the like. These materials are foods or additives for foods and accordingly particularly preferable from the standpoint of safety.

From the experimental results, it is preferred that the antioxidant is used in a proportion of 0.2 to 1 % by weight to cinnamic aldehyde. In the case of eugenol capable of giving a sufficient oxidation inhibiting action in a proportion of at most 1 %, in particular, the addition thereof in a proportion exceeding 1 % is meaningless and it is rather found that such

a higher concentration offsets the microbe inhibiting effect of cinnamic aldehyde.

The composition of the present invention is preferably in such a form as to be sprayed on crops in storage as well as practical use and it is more preferable to convert the composition into an emulsion or solid supported on a support, for example, powder or granules. The preferable embodiments of the present invention will now be illustrated in the case of an emulsion and solid.

The emulsion of the present invention can be prepared by adding water and an emulsifier to cinnamic aldehyde and an antioxidant and then homogenizing the resulting mixture. As the antioxidant for this emulsion, there can preferably be used vitamin E, n-propyl gallate, BHT, eugenol, etc. Above all, vitamin E has a higher oxidation inhibiting effect.

Useful examples of the emulsifier are those of anionic type such as fatty acid salts, higher alcohol sulfuric esters and alkylallylsulfonates; those of nonionic type such as polyoxyethylene alkyl ethers, polyoxyethylene alkylphenyl ethers, polyoxyethylene alkyl esters, polyoxyethylene sorbitan alkyl esters and sorbitan alkyl esters; and those of cationic type such as aliphatic amino salts, quaternary ammonium salts and alkylpyridinium salts. These emulsifiers can be used individually or in combination.

Preferably, the emulsion used in the present invention comprises 5 to 50 % by weight of cinnamic aldehyde, 0.01

to 0.5 % by weight of an antioxidant, 0.5 to 10 % by weight of an emulsifier and the balance water. For practical use in a field, a reagent or preparation containing 5 to 50 % by weight of cinnamic aldehyde is preferable.

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Examples of the emulsion composition of the present invention are given in the following without limiting the present invention. L-64 and P-102 are condensates of polyoxyethylene-polyoxypropylene, commercially available as commercial names Pluronic L-64 and Pluronic P-102 manufactured by Asahi Denka Co., Ltd.

Composition 1

Cinnamic Aldehyde	400 g
Vitamine-E 80	4 g
L-64	50 g
P-102	50 g
Warm Water	<u>496 g</u>
	1000 g

Composition 2

Cinnamic Aldehyde	200 g
BHT	2 g
L-64	50 g
P-102	50 g
Warm Water	<u>698 g</u>
	1000 g

Composition 3

Cinnamic Aldehyde	400 g
n-Propyl Gallate	4 g
Polyoxyethylene Hardened Castor Oil	50 g
Warm Water	<u>546 g</u>
	1000 g

Composition 4

Cinnamic Aldehyde	200 g
Eugenol	2 g
L-64	50 g
P-102	50 g
Warm Water	<u>698 g</u>
	1000 g

The composition of solid type according to the present invention can be obtained by supporting cinnamic aldehyde and an antioxidant on a support. As the antioxidant, for example, there can be used vitamine E, BHT, n-propyl gallate, eugenol, etc. Above all, eugenol having a higher oxidation inhibiting effect is preferably used.

As the support of the present invention, there are for example used porous inorganic materials for adsorption, such as calcium oxide, silicon oxide, magnesium oxide, aluminum oxide, montmorillonite, bentonite, zeolite, white carbon, calcium silicate, etc. In particular, white carbon and calcium silicate are preferable. Calcium silicate fine powder is capable of adsorbing cinnamic aldehyde in an amount of four times

as much as the weight of the fine powder, while white carbon adsorbes at most 1.5 times. In practical use in a field, the concentration of cinnamic aldehyde is 5 to 50 % by weight.

5 This concentration is sufficient and no higher concentration is required, so white carbon with a relatively low adsorptive capacity can sufficiently be used.

Examples of the solid composition of the present invention are given in the following without limiting the present invention.

10 Composition 5

Cinnamic Aldehyde	100 g
White Carbon	200 g
Eugenol	1 g
	301 g

15 Composition 6

Cinnamic Aldehyde	600 g
White Carbon	394 g
Eugenol	6 g
	1000 g

20 Composition 7

Cinnamic Aldehyde	300 g
White Carbon	685 g
Eugenol	15 g
	1000 g

25 Details of the action and advantages by the combination of cinnamic aldehyde and antioxidants, experiments relating

to the effects of antioxidants, processes for the production of the composition according to the present invention and methods of applying the composition to crops will now be illustrated:

5 It is to be noted that there is a large difference in oxidation inhibiting effect between both the cases of using an emulsion composition and solid composition, even if using a same antioxidant. As is evident from the following results of Experiments 1 and 2 shown in Table 1 and Table 4, in the case of emulsions, the effects of vitamine E, BHT, n-propyl gallate and eugenol are higher, while in the case of solid compositions, eugenol shows a higher effect. That is, eugenol shows a higher oxidation inhibiting effect in both the cases and this effect is sufficient by the addition thereof in a proportion of 1 % by weight to cinnamic aldehyde. The addition of more than 1 % by weight is not only meaningless, but a higher concentration rather offsets the microbe inhibiting effect of cinnamic aldehyde. Vitamine E shows a higher oxidation inhibiting effect in the case of emulsions, but a lower effect in the case of solid compositions. Therefore, it is necessary to carefully select a suitable antioxidant depending on the type of the composition to be applied.

The following examples are given in order to illustrate the present invention in detail without limiting the same.

Examples

Experiment 1

1000 g of an emulsion comprising 200 g of cinnamic aldehyde, 40 g of a surfactant, 0.4 g of each of the following antioxidants (1) to (5) (0.2 % by weight to cinnamic aldehyde) and the balance of water was prepared, while 1000 g of a comparative emulsion comprising 200 g of cinnamic aldehyde, 40 g of the surfactant and the balance of water, being free from the antioxidant, was prepared, As the antioxidant, there were used commercially available food additives, i.e. (1) dibutylhydroxytoluene (commercial name, BHT manufactured by Takeda Yakuhin Co., Ltd.), (2) licorice extract (commercial name, Sankanon manufactured by Maruzen Kasei Co., Ltd.), (3) vitamin E (commercial name E-80, containing 80 % of natural vitamin E, manufactured by Eisai Co., Ltd.) (4) n-propyl gallate (Wako Junyaku Co., Ltd.) and (5) L-ascorbyl stearate (Tokyo Kasei Co., Ltd.).

After the above described emulsions were allowed to stand at room temperature for 16 days, analysis of the components was carried out by gas chromatography to determine the oxidation inhibiting effects of the antioxidants (1) to (5) for cinnamic aldehyde. The purity of the cinnamic aldehyde used was 97.6 % of cinnamic aldehyde and 0.4 % of cinnamic acid, as a result of measurement by gas chromatography. The experimental results are as shown in Table 1, in which the order of the oxidation inhibiting effects from higher to lower is vitamin E, eugenol, n-propyl gallate and BHT, and Sankanon has little effect whereas L-ascorbyl stearate reversely promotes the

oxidation.

Table 1 Oxidation Inhibiting Effect of Cinnamic Aldehyde Emulsion (wt %)

	<u>Antioxidant</u>	<u>Amount</u>	<u>Cinnamic Aldehyde</u>	<u>Cinnamic Acid</u>
5	BHT	0.2 %	95.8 %	1.2 %
	Sankanon	-do-	71.4 %	21.0 %
	Vitamine E-80	-do-	97.2 %	0.4 %
	Eugenol	-do-	96.8 %	0.8 %
	n-Propyl Gallate	-do-	95.9 %	1.0 %
10	L-Ascorbyl Stearate	-do-	65.1 %	26.3 %
	Comparison	-	70.4 %	20.1 %

Example 1

Emulsion A of the present invention and antioxidant-free Emulsion B of the prior art were prepared by metering the following compositions and emulsifying by means of a homogenizer, based on the results of Experiment 1, and then subjected to a Schale test in a room, thus obtaining results as shown in Table 2.

Composition of Emulsion A of
the present invention (wt %)

20	Cinnamic Aldehyde	20
	BHT	1
	Pluronic L-64	5
	Pluronic P-102	5
25	Warm Water	69

Composition of Emulsion B of
the Prior Art (wt %)

Cinnamic Aldehyde	20
Pluronic L-64	5
Pluronic P-102	5
Warm Water	70

Test culture media were prepared by adding Emulsions A and B to a PSA culture medium to give concentrations of 200 ppm and 400 ppm respectively, adequately shaking and pouring in petri dishes. PSA is the abbreviation for potato, sucrose and agar which is known as a potato culture medium. Each of microorganisms shown in Table 2 which had previously been cultured in other culture media was cut into 5 mm square and incubated in the center of the each solidified culture medium. Judgment of the effects of Emulsions A and B was carried out by subjecting the petri dishes for testing to culturing at 25 °C for 6 days and metering the growth diameter (mm) so as to know the growth inhibiting effect on the mycelial colony. A grown mycelial colony diameter of 70.0 mm, corresponding to the Schale with a diameter of 70 mm, means that the Schale was full of the mycelial colony.

Summarizing the results of Table 2, there was found no large difference before the passage of 6 days from the start of the test between Emulsions A and B, but the difference appeared and increased gradually with the passage of time and Emulsion B lost its effect 30 days after the start of the test

as a result of the final test, whereas Emulsion A maintained
its effect for the same time of period. These results appar-
ently teach that according to the present invention, the oxida-
tion of cinnamic aldehyde can effectively be prevented and the
5 intrinsic microbe inhibiting effect thereof can be maintained.

**Table 2 Test of Microbe Inhibiting Effect of Cinnamic
Aldehyde Emulsions A and B**

Microbes Tested	Concentration of Emulsion	6 days	10 days	15 days	20 days	30 days
Rizoctonia solani	A 200 ppm	-	-	-	5.0	8.5
	B 200 ppm	-	16.0	26.0	50.8	70.0
	A 400 ppm	-	-	-	-	-
	B 400 ppm	-	-	17.0	39.0	70.0
Pythium debaryanum	A 200 ppm	-	-	-	-	-
	B 200 ppm	-	10.5	18.0	35.0	70.0
	A 400 ppm	-	-	-	-	-
	B 400 ppm	-	-	22.0	39.0	70.0
Collectotri- chum lagen- arium	A 200 ppm	-	-	6.0	10.2	22.0
	B 200 ppm	20.6	39.0	70.0	70.0	70.0
	A 400 ppm	-	-	-	-	-
	B 400 ppm	-	-	-	-	-
Fusarium o- xysporum f, cucumerinum	A 200 ppm	-	-	-	-	7.8
	B 200 ppm	-	4.5	11.0	30.7	70.0
	A 400 ppm	-	-	-	-	-
	B 400 ppm	-	-	-	-	10.0
Botrytis cinerea	A 200 ppm	-	-	-	-	-
	B 200 ppm	-	10.2	40.5	70.0	70.0
	A 400 ppm	-	-	-	8.0	30.0
	B 400 ppm	-	-	16.5	70.0	70.0

(mm)

Example 2

As to Emulsions A and B of Example 1, a microbe inhibiting test in a soil was conducted. Cucumber seedlings with developed fifth leaves were transplanted in a contaminated soil with *Fusarium oxysporum f cucumerinum* to prepare four areas with three repetitions (total twelve spots), i.e. Emulsion A Area, Emulsion B Area, Benomyl Area and Non-treated Area. The contaminated soil contained *Fusarium* microbes in a proportion of 10^5 per 1 g of the dry soil.

Immediately after the transplantation, each of the emulsions with components concentration adjusted to 400 ppm was irrigated into the soil in a rate of 3000 ml per m^2 to give pots with a diameter of 10 cm and subjected to examination of affected seedlings after 30 days, thus obtaining results shown in Table 3. As apparent from Table 3, in the case of the anti-oxidant-free Emulsion B, the microbe inhibiting effect was greatly lowered, while in the case of Emulsion A according to the present invention, the microbe inhibiting effect was maintained even after 30 days:

Table 3 Pot Test Results on Cucumber with Cinnamic Aldehyde Emulsions A and B

<u>Reagents</u>	<u>Concentration (Components)</u>	<u>Test Seedlings</u>	<u>Affected Seedlings</u>	<u>Affection Percent</u>	<u>Chemical Damage</u>
Emulsion A	400 ppm	12	0	0 %	-
Emulsion B	400 ppm	12	3	25 %	-
Benomyl Compound	500 ppm	12	1	8 %	-
Non-treated	-	12	12	100 %	-

Experiment 2

In this experiment, fine powder of white carbon (commercial name, Tokusil N, manufactured by Tokuyama Soda Co., Ltd.) was used as a support. Preparation of a sample was carried out by charging a polyethylene bag having a volume of 1000 ml with 10 g of white carbon and 10 g of cinnamic aldehyde (purity: 98.1 %) mixed with a predetermined quantity of an antioxidant and mixing them adequately. On the other hand, a control sample was prepared by similarly adsorbing 10 g of cinnamic aldehyde on 10 g of white carbon.

Each of the thus prepared samples in an amount of 20 g was charged in a wide-mouthed transparent bottle of polyethylene with a volume of 1000 ml and allowed to stand in an open thermostat maintained at 40 °C for 14 days, during which the bottle was shaken several times a day so as to bring the sample in the bottle into uniform contact with the air. After 14 days, 3 g of the sample was weighed, subjected to extraction with 100 ml of ether by means of a Soxhlet extractor, treated with diazomethane after distilling off the ether, and then subjected to methyl-esterification of cinnamic acid, which was then determined quantitatively by gas chromatography. The results are shown in Table 4.

It is apparent from the results of Table 4 that eugenol has a higher oxidation inhibiting effect, but Vitamin E 80 and EG-5DX (commercial name, manufactured by Nippon Yushi Co., Ltd., composition: vitamin-E 30 %, ethyl alcohol 15 %,

vitamin-C 0.1 %, plant fats and oils, gallaic acid 54.9 %) has little effect.

Table 4 Oxidation Inhibiting Effect of Cinnamic Aldehyde Composition of Solid Type

Antioxidant	Amount	Cinnamic Aldehyde	Cinnamic Acid
Control	-	4.16 %	67.05 %
Eugenol	1 %	92.67 %	2.05 %
-do-	3 %	90.61 %	1.25 %
EG-5DX	1 %	9.84 %	64.09 %
-do-	2 %	3.15 %	61.61 %
Vitamin-E 80	1 %	9.84 %	64.09 %

The following experiment was carried out to examine how nematodes living in the soil are affected by a composition comprising cinnamic aldehyde and eugenol, as an antioxidant, in a proportion of 1 %, adsorbed on white carbon, and another composition comprising cinnamic aldehyde adsorbed on white carbon. As the nematode, there were chosen *Meloidogyne incognita* and *Steternema jeltiae*. The former is a noxious insect greatly damaging crops and the latter is a non-toxic self-supporting nematode. As well known in the art, such a well balance in the soil serves to protect the roots of crops that the density of the former is small and that of the latter is large.

Experiment 3

A powdered composition comprising cinnamic aldehyde adsorbed on white carbon was divided into four areas with

cinnamic aldehyde concentrations of 125 ppm, 250 ppm, 500 ppm and 1000 ppm with provision of a control area free from cinnamic aldehyde.

100 nematodes of *Meloidogyne incognita* were immersed in aqueous dispersion of these compositions for 1 hour, washed with distilled water and then immersed in distilled water for 24 hours. Then, the number of the surviving ones was examined to give the following results:

<u>Concentration Area</u>	<u>Surviving Nematodes</u>
125 ppm area	90
250 ppm area	69
500 ppm area	0
1000 ppm area	0
Control area	98

When the above procedure was repeated except adding 1 % of eugenol to the cinnamic aldehyde, the following results were obtained:

<u>Concentration Area</u>	<u>Surviving Nematodes</u>
125 ppm area	90
250 ppm area	58
500 ppm area	0
1000 ppm area	0

Examples 3 and 4

The following tests were carried out to known the effects in the soil for a long time.

In a nematode soil test A (Example 3), three samples

were prepared by adding 1 % by weight of eugenol to cinnamic aldehyde and allowing to adsorb on white carbon respectively to give concentrations of 20 %, 33 % and 56 % by weight. 8 g of each of the thus resulting samples was added to 400 ml of water to form a suspension, and irrigated in 10 l of a soil contaminated with *Meloidogyne incognita* so as to constantly maintain a soil humidity of 30 %, thus providing four test areas including a non-added control area, as shown in Table 5.

In a nematode soil test B (Example 4), three comparative samples were prepared by allowing cinnamic aldehyde to adsorb on white carbon respectively to give concentrations of 20 %, 33 % and 66 % by weight and then applied to the soil in the similar manner to described above, thus providing four test areas including a non-added control area, as shown in Table 6.

In both the tests, the number of *Meloidogyne incognita* and *Steinernema jeltiae* was examined after 4 days and 30 days by Baermann funnel method and consequently, it was found that in Tests A and B, there were no significant differences among the test areas after 4 days, but in the examination after 30 days, the samples containing eugenol in addition to cinnamic aldehyde in Test A gave apparently more excellent effects.

In Tables 5 and 6, M represents *Meloidogyne incognita* and S represents *Steinernema jeltiae*.

11 4 00 14

Table - 5 Nematode Soil Test A Cinnamic Aldehyde + Eugenol + White Carbon

		Cinnamic Aldehyde Concentration	Nematode	No. 1	No. 2	No. 3	No. 4	Total	Average
Four Days	Control		M	162	163	124	76	525	131.25
			S	219	106	106	128	559	139.75
	20 %		M	0	8	16	5	29	7.25
			S	458	405	468	177	1508	377.0
	33 %		M	2	0	3	3	8	2.0
			S	143	158	206	120	627	156.75
	66 %		M	0	2	0	0	2	0.5
			S	42	58	41	97	258	64.5
Thirty Days	Control		M	24	7	11	6	48	12.0
			S	173	96	77	81	427	106.75
	20 %		M	2	2	1	3	8	2.0
			S	202	181	131	231	750	187.5
	33 %		M	0	0	0	1	1	0.25
			S	142	154	152	217	665	166.25
	66 %		M	0	0	0	0	0	0
			S	258	31	116	66	471	117.75

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Table 6

Cinnamic Aldehyde Concentration		Nematode	No. 1	No. 2	No. 3	No. 4	Total	Average
Four Days	Control	M	361	266	108	120	855	213.55
		S	416	384	193	209	1202	300.5
	20 %	M	109	206	250	166	731	182.75
		S	347	487	735	544	2112	528.0
	33 %	M	113	186	210	169	678	169.5
		S	324	617	707	528	2176	544.0
	66 %	M	203	288	270	175	936	234.0
		S	449	630	599	350	2028	507.0
	Control	M	55	41	20	186	302	75.5
		S	123	94	65	111	393	98.25
	20 %	M	451	54	47	50	602	150.5
		S	101	114	93	106	414	103.5
Thirty Days	33 %	M	118	128	66	47	359	89.75
		S	131	206	98	79	514	128.5
	66 %	M	1095	684	828	219	2826	706.5
		S	175	242	200	60	677	169.25

It is known that eugenol has an oxidation inhibiting effect. However, there have been published no literatures as to how much eugenol or other antioxidants are to be added to cinnamic aldehyde in order to inhibit the oxidation of cinnamic aldehyde and to maintain the intrinsic microbe inhibiting effect.

The inventor has a number of tests so as to solve the above described problem and consequently, have found that addition of antioxidants, in particular, eugenol in a proportion of 1 to 5 % by weight to cinnamic aldehyde results in inhibition of the oxidation of cinnamic aldehyde, but with the increase of the addition quantity thereof in the range of more than 1 % by weight, the microbe inhibiting effect is rather deteriorated. This is a completely unexpected result, because it has hitherto been reported that the phenol coefficients for grampositive microbes fluctuate to some extent depending upon analyzers, but there is not a large difference in microbe inhibiting effect. That is, cinnamic aldehyde gives a microbe inhibiting effect of 17, while eugenol, 15 ((1) A. R. Penfold: "Perfume and Essential Oil Report" 15, p 388 (1925)), cinnamic aldehyde gives a microbe inhibiting effect of 3.0, while eugenol gives that of 8.5 ((2) S. Lideal: *ibid*, 19, p 285 (1928)) and cinnamic aldehyde gives a microbe inhibiting effect of 8.8 while eugenol gives that of 14.4 ((3) H. Flueeller: "Seifen Ehre Fette Wachse" 98 p 677 (1972)).

The inventor has made examinations on methods of

protecting plants, in particular, crops. Thus, it is found that there is a remarkable difference with respect to the inhibiting effect of gram-negative microbes which damage crops between cinnamic aldehyde and eugenol and the eugenol to cinnamic aldehyde ratio of the microbe inhibiting effect is in the range of 1/5 to 1/10. That is, the microbe inhibiting action or effect against gram-negative microbes is completely unexpected from the microbe inhibiting action or effect against gram-positive microbes, as described in the above literatures (1) to (3). In both of a Schale test in a room (Experiment 4) and a pot test using cucumber (Experiment 5), the microbe inhibiting effect is relatively lowered with the increase of the amount of eugenol.

Experiment 4 (Schale Test)

To 60 g of white carbon were added cinnamic aldehyde and eugenol in proportions as shown in the following table to prepare seven sample compositions differing in concentration. As microbes to be tested, five popular microbes which attack crops were chosen and subjected to testing by preparing PSA culture media from the sample compositions to give cinnamic aldehyde concentrations of 25, 50, 100 and 200 ppm. The microbe inhibiting effects were compared by the grown diameters of mycelial colony. Using a Schale with a diameter of 70 mm, the data of 70 mm means that the Schale was full of the mycelial colony. Data obtained by determining three days and five days after inoculation of the microbes for check are shown in

Table 7.

1) Sample Compositions (weight ratio)

		Cinnamic Aldehyde	Eugenol	Eugenol/Cinnamic Aldehyde Ratio(wt%)	White Carbon
5	TE-1	39.6	0.4	1	60
	TE-2	39.2	0.8	2	60
	TE-5	38.0	2.0	5.2	60
	TE-10	36.0	4.0	11	60
	TE-20	32.0	8.0	25	60
	TE-40	24.0	16.0	66	60
	TE-50	20.0	20.0	100	60

2) Microbes Tested

Fusarium oxysporum f. cucumerinum, Pythium debaryanum,
Botrytis cinerea, Rhizoctonia solani, Colletotrichum
lagenarium

3) Concentrations Tested

25, 50, 100 and 200 ppm

4) Test Method

Testing on PSA plate medium

Table - 7

	Microbes Conc. (ppm)	3 Days					5 days				
		I	II	III	IV	V	I	II	III	IV	V
TE - 1	25	20	70	68	37	62	54	70	70	65	70
	50	10	70	50	34	57	46	70	70	63	70
	100	0	70	15	32	47	40	70	65	53	70
	200	0	0	0	0	0	0	0	0	0	0
TE - 2	25	20	70	68	38	62	55	70	70	65	70
	50	10	70	50	36	57	48	70	70	63	70
	100	5	70	17	32	48	40	70	68	54	70
	200	0	0	0	0	0	0	0	0	0	0
TE - 5	25	22	70	68	40	63	58	70	70	66	70
	50	8	70	55	37	59	50	70	70	63	70
	100	5	70	20	33	53	41	70	70	57	70
	200	0	0	0	0	0	0	0	0	0	0
TE - 10	25	30	70	70	40	63	60	70	70	65	70
	50	25	70	70	38	60	53	70	70	64	70
	100	13	70	50	37	48	45	70	70	59	70
	200	10	8	10	10	5	20	40	60	40	60
TE - 20	25	39	70	70	40	63	60	70	70	66	70
	50	35	70	70	38	60	56	70	70	65	70
	100	30	70	70	37	50	50	70	70	63	70
	200	23	15	15	20	40	45	60	68	45	67
TE - 40	25	40	70	70	40	64	63	70	70	66	70
	50	38	70	70	37	62	60	70	70	66	70
	100	34	70	70	37	55	57	70	70	64	70
	200	26	30	17	33	47	52	70	70	52	70
TE - 50	25	42	70	70	42	64	65	70	70	66	70
	50	40	70	70	38	62	60	70	70	65	70
	100	38	70	70	37	55	58	70	70	62	70
	200	30	60	20	34	48	55	70	70	53	70
	0	43	70	70	42	64	69	70	70	66	70

Note: I: Fusarium, II: Pythium; III: Rhizoctonia;
IV: Colletotrichum; V: Botrytis

Experiment 5 (Pot Tests A and B)

Using the same sample compositions as used in Experiment 4, pot tests were carried out on soil pathogenic microbes, i.e. Pythium and Fusarium. Dilution of the sample composition was 1000 times and cucumber (Tokiwa Hikari No. 3, P-type) was used as an object crop. That is, a soil contaminated with each of the pathogenic microbes was charged in a 1/5000 are Wagner pot and immediately after seeding a germinating seed of cucumber therein, the each sample composition was irrigated at a rate of 3000 ml/m², after which the same amount thereof was irrigated every 10 days and examination was conducted 30 days after seeding, thus obtaining results shown in Tables 8 and 9. In any of the test areas, better results are obtained in the case of adding eugenol in a proportion of 1 to 5 % by weight to cinnamic aldehyde and the effect of eugenol is rather lowered with the further increase of the amount thereof.

Table 8 Results of Pot Test A (Fusarium)

Sample Composition	Multiple of Dilution	Seedlings Tested	Seedlings Affected	Seedlings Affected(%)
TE 1	1000	50	0	0
TE-2	1000	50	0	0
TE-5	1000	50	1	2
TE-10	1000	50	10	20
TE-20	1000	50	19	38
TE-40	1000	50	27	54
TE-50	1000	50	30	60
Non-treated	-	50	35	70

Table 9 Results of Pot Test B (Pythium)

Sample Composition	Multiple of Dilution	Seedlings Tested	Seedlings Affected	Seedlings Affected(%)
TE-1	1000	50	0	0
TE-2	1000	50	0	0
TE-5	1000	50	0	0
TE-10	1000	50	5	10
TE-20	1000	50	18	36
TE-40	1000	50	33	66
TE-50	1000	50	41	82
Non-treated	-	50	46	92

Example 5

Using a cinnamic aldehyde wettable powder of the present invention comprising 39.6 % by weight of cinnamic aldehyde, 0.4 % by weight of eugenol and 60.0 % by weight of white carbon (referred hereinafter to as TM wettable powder), a test on the scab of Japanese apricot was carried out by the following test method, thus obtaining results as shown in Table 10. It is apparent from this result that the TM wettable powder diluted with a multiple of 100 times gives a more excellent insect exterminating effect than a control composition without affecting leaves and fruits.

Test Method

Test Place: Miyakawauchi, Donaricho, Edanogun

Kind and Tree Age: Uguisu Yado, 7 years

Scale of Test: one tree per one plot and four

replication

Diseases and insects for check: somewhat many

Spraying Before Test: no

Treatment Time, Amount and Method: A sufficient amount

5 of the wettable powder was sprayed by means of a power sprayer in such a manner that the liquid fell in drops four times on April 28, May 9, May 20 and May 27.

10 Weather during Testing Period: The atmospheric temperature from April to May was higher than the normal year. The rainfall during the same period was more than the normal year.

15 Examination Date and Method: The examination was conducted June 3, 1986 as to whether there had occurred disease of 200 fruits per one tree and damages from the chemicals.

Table 10 Results of Test on Scab of Japanese Apricot

Sample Composition	Content (%)	Concentration Dilution Multiple	Effec- tive Con- tent (ppm)	Fruits Number Exam- ined	Disease Ratio (%)	Damage by Chem- icals (%)	Utili- ty
Present Invention							
TM Wettable Powder	40	500	800	800	4.0	—	yes
For Comparison							
Topzine M Wettable Powder	70	1500	467	800	6.7	—	
Blank	No Spraying			850	51.6		

The preceding examples and experiments are in no way to be construed as limiting the scope of the present invention, these specific embodiments being presented simply to exemplify the best mode of operation of the disclosed method.

It will clearly be understood from the above described examples and experiments that according to the method of protecting crops of the present invention, there can be obtained following advantages or merits. That is, the use of a non-toxic and stable composition comprising non-toxic and harmless cinnamic aldehyde and an antioxidant results in a selectively strong microbe inhibiting effect against, in particular, pathogenic microbes or molds in the soil without having no influences upon useful microorganisms, and in a breeding effect of harmless nematoda, for example, *Steinernema feltiae* in spite of inhibiting noxious *Meloidogyne incognita*. This is a more excellent method of protecting crops, whereby the antibiosis by microorganism can effectively be utilized so as to advantage protection of crops and such effects as described above can stably be maintained by the presence of an antioxidant. Furthermore, this method serves to improve the soils without unfavorably affecting the human body and environment and is advantageous from an economical point of view.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A method of protecting crops against attack by insect pests, microorganisms and pathogenic microbes, the method comprising the application to the crops of a composition comprising cinnamic aldehyde and an antioxidant.
2. The method as claimed in claim 1, wherein the composition further contains an emulsifier.
3. The method as claimed in claim 2, wherein the emulsifier is at least one selected from the group consisting of fatty acid salts, higher alcohol sulfuric acid esters, alkylallylsulfonates, polyoxyethylene alkyl ethers, polyoxyethylene alkylpheno ethers, polyoxyethylene alkyl esters, polyoxyethylene sorbitan alkyl esters, sorbitan alkyl esters, aliphatic amino salts, quaternary ammonium salts and alkylpyridinium salts.
4. The method as claimed in any preceding claim, wherein the composition is supported on a support.
5. The method as claimed in claim 4, wherein the support is of a material selected from the group consisting of calcium oxide, silicon oxide, magnesium oxide, aluminum oxide, montmorillonite, bentonite, zeolite, white carbon and calcium silicate.
6. The method as claimed in claim 4 or 5, wherein the cinnamic aldehyde is supported on the support in a proportion of 5 to 50 % by weight.
7. The method as claimed in any preceding claim, wherein the antioxidant is at least one selected from the



group consisting of vitamin E, n-propyl gallate, BHT and eugenol.

8. The method as claimed in any preceding claim, wherein the antioxidant is in a proportion of 0.2 to 1 % by weight relative to the cinnamic aldehyde.

9. The method as claimed in claim 1, wherein the composition comprises 5 to 50 % by weight of cinnamic aldehyde, 0.01 to 0.5 % by weight of an antioxidant, 0.5 to 10 % of an emulsifier and the balance of water.

10. A composition for protecting crops against attack by insect pests, microorganisms and pathogenic microbes, said composition comprising cinnamic aldehyde and an antioxidant, and optionally an emulsifier or a support.

11. A method according to claim 1, or a composition according to claim 10, substantially as hereinbefore described with reference to the Examples.

DATED this 5th day of February 1991.

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