

Patents Act 1990

PATENT REQUEST: STANDARD PATENT/PATENT OF ADDITION

We, being the person(s) identified below as the Applicant, request the grant of a patent to the person identified below as the Nominated Person, for an invention described in the accompanying standard complete specification.

Full application details follow.

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[54] Invention Title: USE OF 1-HYDROXYMETHYLPYRAZOLES AS AGRICULTURAL MICROBICIDES

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BASIC CONVENTION APPLICATION(S) DETAILS

Table with 4 columns: [31] Application Number, [33] Country, Country Code, [32] Date of Application. Row 1: 555,035, United States of, US, 13th July 1990

TICK IF APPLICABLE

For the purposes of Section 40, the specification relies on Section 6 of the Act (Microorganisms).

Drawing number recommended to accompany the abstract

By our/my Patent Attorneys, WATERMARK PATENT & TRADEMARK ATTORNEYS

M 020: 20791

Ian A. Scott
Registered Patent Attorney

11th July 1991.

(Date)

AUSTRALIA

Patents Act 1990

NOTICE OF ENTITLEMENT

(To be filed before acceptance)

We, **BUCKMAN LABORATORIES INTERNATIONAL, INC.** of 1256 North McLean Boulevard, Memphis, Tennessee 38108-0305, United States of America being the applicant in respect of Application No. 80422/19 state the following:-

The Person nominated for the grant of the patent has entitlement from the actual inventors by assignment.

The person nominated for the grant of the patent has entitlement from the applicant of the basic application listed on the patent request form by assignment.

The basic application listed on the request form is the first application made in a Convention country in respect of the invention.

BUCKMAN LABORATORIES INTERNATIONAL, INC.

By our Patent Attorneys,

WATERMARK PATENT & TRADEMARK ATTORNEYS



.....
Ian A. Scott

Registered Patent Attorney

23rd December, 1993

.....
(Date)

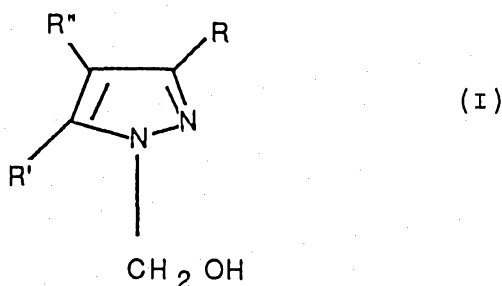


AU9180422

(12) PATENT ABRIDGMENT (11) Document No. AU-B-80422/91
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 647496

- (54) Title
USE OF 1-HYDROXYMETHYLPYRAZOLES AS AGRICULTURAL MICROBICIDES
- International Patent Classification(s)
(51)⁵ **A01N 043/56**
- (21) Application No. : 80422/91 (22) Application Date : 12.07.91
- (30) Priority Data
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555035 13.07.90 US UNITED STATES OF AMERICA
- (43) Publication Date : 16.01.92
- (44) Publication Date of Accepted Application : 24.03.94
- (71) Applicant(s)
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- (56) Prior Art Documents
EP 320309
- (57) Claim

1. A method for controlling microorganisms on a plant comprising applying to said plant or the seed thereof an amount sufficient to inhibit the growth and proliferation of at least one microorganism on said plant or seed of a compound of the formula I



wherein R and R' are independently selected from hydrogen and an alkyl of 1 to 4 carbon atoms, and R'' is a moiety selected from the group consisting of (a) hydrogen, (b) a halogen atom selected from the group consisting of F, Cl, Br and τ , and (c) a nitro group, or the hydrochloride salt of said compound.

647496

Form 10

COMMONWEALTH OF AUSTRALIA
PATENTS ACT 1952-69

COMPLETE SPECIFICATION

(ORIGINAL)

Class

Int. Class

Application Number:
Lodged:

Complete Specification Lodged:
Accepted:
Published:

Priority :

Related Art :

Name of Applicant :

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Complete Specification for the invention entitled:

USE OF 1-HYDROXYMETHYLPYRAZOLES AS AGRICULTURAL MICROBICIDES

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

CONTROL OF MICROORGANISMS ON PLANTS
WITH 1-HYDROXYMETHYLPYRAZOLES

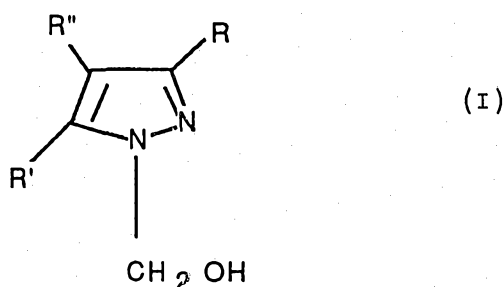
BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

5 The subject of the present invention is directed to a method and composition for the control of microorganisms on plants. More particularly, the present invention relates to the use of certain 1-hydroxymethylpyrazoles as an agricultural microbicide, especially as a bactericide or fungicide.

SUMMARY OF THE INVENTION

10 The present invention provides a method for the control of microorganisms, including but not limited to bacteria and fungi, comprising the step of applying to a plant or the seed thereof an amount sufficient to inhibit the growth and proliferation of at least one microorganism on the plant or seed of a compound having the formula I



wherein R and R' are independently selected from hydrogen and alkyl of 1 to 4 carbon atoms, R'' is a moiety selected from the group consisting of (a) hydrogen, (b) a halogen atom selected from the group consisting of F, Cl, Br and I, and (c) a nitro group, or the hydrochloride salt of said compound.

20 The applying step may be accomplished by spraying a composition comprising a compound of formula I. A sufficient amount of the compound of the formula I can be in the range of about 2,500 to about 30,000 ppm of the composition, and preferably in the range of about 15,000 to about 25,000 ppm of the composition. The compound can be 3,5-dimethyl-1-hydroxymethylpyrazole or 3,5-dimethyl-1-hydroxymethylpyrazole hydrochloride. The microorganisms can be bacteria and fungi. The bacteria can be Erwinia caratovora, Agrobacteria sp., corynebacteria, Xanthomonas sp., Pseudomonas sp., Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. The fungi can be Pythium ultimum, Helminthosporium sativum, Rhizoctonia solani, Monilia

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fructicola, *Fusarium oxysporum*, *Peronospora parasitica* and *Alternaria solani*. The plant or seed can be ornamental plants, fruit plants, vegetable plants, cereal crop plants and seeds thereof. The application of the compound or hydrochloride salt to the plant or seed can be done two or more times.

5 A composition is also provided which includes an amount per unit weight of the compound of formula I sufficient to inhibit the growth and proliferation of at least one microorganism on the plant, together with an inert carrier. The compound can be 3,5-dimethyl-1-hydroxymethylpyrazole or 3,5-dimethyl-1-hydroxymethylpyrazole hydrochloride. The composition can include at least one inert additive relative to the
 10 compound of formula I, and the additive can be from stabilizers, spreading agents, wetting agents, sticking agents, fillers, bactericides, fungicides, pesticides, and any combination thereof. The combination can be in the form of a solution or dispersion with the carrier being inert relative to the compound of formula I, and can be liquid and powders. For example, the carrier can be water or a flowable power.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

For purposes of the present invention, the term "microorganism" is intended to include but is not limited to bacteria, fungi and yeasts, and is intended to include but is not limited to phytopathogens within these families.

The method and composition of the invention may be used to prevent attacks by at
 20 least one microorganism on plants, including but not limited to ornamental plants, fruit plants, vegetable plants and cereal crop plants and seeds thereof. Ornamental plants can include orchids and roses; fruit plants can include orange trees and pear trees; vegetable plants can include cauliflower, tomatoes and onion plants. Bactericidally or fungicidally effective amounts of the compound of formula I can be alternatively applied
 25 to the bacteria, the fungi or the respective hosts, particularly hosts like plants, plant seeds, paper and the like, which are attacked by these microorganisms.

The bactericidal and fungicidal activities of the compounds of the invention are surprising insofar as N-methyl compounds of similar stability, for example, N-hydroxymethylphthalimide, are not very effective with regard to their antimicrobial and preservative activity.
 30

The compounds of this invention may be prepared by reacting either solid paraformaldehyde or an aqueous formaldehyde solution with a pyrazole derivative. Preferred methods of preparation are given in Dvoretzky et al., Formaldehyde Condensation in the Pyrazole Series, 15 Journ. Org. Chem. 1285-8 (1952) and Huttel
 35 et al., Mannische Reaktion der Pyrazole, 85 Chemische Berichte 820-26 (1952),

both hereby specifically incorporated by reference. Another preferred method of preparation is given in an application of Fenyes and Pulido entitled "Process for the Preparation of 1-Hydroxymethylpyrazoles", filed July 20, 1990, concurrently herewith, which is hereby specifically incorporated by reference.

5 The antimicrobial activity of the compounds used in accordance with the invention extends to a variety of different microorganisms, including but not limited to bacteria such as *Erwinia caratovora*, *Agrobacteria* sp., *corynebacteria*, *Xanthomonas* sp., *Pseudomonas* sp., *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and including but not limited to fungi such as *Phthium ultimum*, *Helminthosporium sativum*, *Rhizoctania solani*, *Fusarium oxysporum*, *Monilia fructicola*, *Peronospora parasitica* and *Alternaria solani*.

10 The preferred compounds of this invention include 3,5-dimethyl-1-hydroxymethylpyrazole, or the hydrochloride salt thereof, which generally exhibit relatively increased bactericidal and fungicidal activity under identical conditions over the 3,5-dimethyl-4-substituted-1-hydroxymethylpyrazoles in accordance with the invention.

15 The pyrazoles of this invention may be formulated and applied in bactericidal or fungicidal amounts, as the case may be, by conventional art methods. The compounds of formula I are preferably used in a composition with one or more inert liquid or solid carriers or excipients such as powders, solutions and dispersions, most preferably in a spray with water or a flowable powder. Compositions of these pyrazoles may also contain compatible stabilizers, spreading agents, wetting agents, sticking agents, fillers, other bactericides, other fungicides, pesticides and the like, and combinations thereof.

20 The concentration of the compounds used in accordance with the invention which inhibits growth and proliferation of at least one microorganism, and thus provides the antimicrobial activity described herein, may be readily determined by one skilled in the art without extensive experimentation. Factors affecting concentration include but are not limited to weather conditions, the type of plant(s) and the type of microorganism(s) present. An effective dosage may possibly be as low as about 2500 parts per million (ppm) (about 0.5% weight per volume water solution basis), will range from about 2500 ppm to about 30,000 ppm (about 3.0%), preferably in the range from about 15,000 (about 1.5%) ppm to about 25,000 ppm (about 2.5%). One or more applications of the compound may be undertaken, as required, with factors affecting frequency of application including but not limited to weather conditions, the type of
25
30
35 plant(s), and the type of microorganism(s) present.

To illustrate the nature of the invention, the following examples are given. It should be understood, however, that the invention is not to be limited to the specific conditions or details set forth in these examples.

EXAMPLE 1

5 The bacterial effectiveness of these compounds was demonstrated in bacterial suspensions prepared by washing a culture of the desired bacteria from agar slant with sterile water into a vessel and further diluting the aqueous suspension to 250 μ L with sterile water. The test was conducted as follows:

10 The pyrazole to be tested was dissolved in acetone to 500 ppm and 30 μ L of this solution was pipetted onto each of two surface areas on a plate covered with 20 mL of Emerson's agar. The treated agar plates were then sprayed with the bacteria suspension and the plates were incubated for 44 to 48 hours at 24°C. The two treated areas of the plate were then observed for bacterial growth. The pyrazole's effectiveness for controlling bacterial growth in the treated areas was rated as "+" or "-"; with the plus sign (+) indicating the areas were completely free of bacterial growth and with the minus sign (-) indicating they were completely overgrown. The results of this comparative bactericidal test appear in Table I.

TABLE I

Effectiveness

20	<u>Compound</u>	Erwinia caratovora	Pseudomonas syringae
15	3,5-dimethyl-1-hydroxy methylpyrazole	+	+
25	3,5-dimethyl-4-chloro-1-hydroxymethylpyrazole	+	+
30	3,5-dimethyl-4-nitro-1-hydroxymethylpyrazole	-	+

EXAMPLE 2

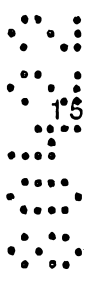
35 The mycelial drop method was used to test fungicidal activity. This method measures the fungitoxicity of a compound in terms of its inhibition of fungus mycelial growth. The general procedure was as follows: Each pyrazole to be tested was dissolved

in acetone to a 100 ppm up to 15,000 ppm concentration. Equal volumes of these solutions were applied to the centers of each of three replicate paper discs inoculated with the desired fungus mycelium and placed on potato-dextrose agar medium.

Following this application, the discs were incubated along with inoculated but untreated control discs at ambient temperatures until the control discs were filled with mycelial growth. The fungicidal activity of the pyrazole was determined by comparing the radii of mycelial growth away from the edges of the treated and control discs. From this comparison a percent inhibition based on the relative mycelial growth areas was determined. The results of this comparative fungicidal test at two selected concentrations appear below in Table II.

EXAMPLE 3

The slide spore germination method was also used to test fungicidal activity. This method is described in the American Phytopathological Society Journal, Vol. 33, pages 627-632 (1943), and measures the fungitoxicity of compounds in terms of their percent inhibition of fungus spores. The general procedure was as follows: Each pyrazole to be tested was dissolved in acetone to several concentrations between 50 ppm up to 15,000 ppm. These solutions were then pipetted into the wells of depression slides and allowed to dry. The wells were filled with a spore suspension of the specified test organism and incubated in a moist chamber overnight. A group of 100 spores was examined and the numbers of spores germinated and not germinated were counted in separate tallies and recorded to show the biological activity in terms of the percentage inhibition. The results of this comparative fungicidal test at two selected concentrations also appear in Table II.



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10

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TABLE II

Percent Inhibition

<u>Concentration</u> (ppm)	<u>Compound</u>	<u>Mycelial Drop</u>			<u>Slide Spore</u>	
		P	H	R	M	A
500	3,5-Dimethyl- 1-hydroxy- methyl- pyrazole	100	97	90	100	100
1000	3,5-Dimethyl- 1-hydroxy- methyl- pyrazole	100	100	100	100	100

P = *Pythium ultimum*H = *Helminthosporium sativum*R = *Rhizoctonia solani*M = *Monilia fruticola*A = *Alternaria solani*

EXAMPLE 4

Other pyrazoles of this invention were tested by the mycelial drop method as described above in Example 2, except that these pyrazoles were dissolved in acetone to a concentration of 500 ppm. The results of this comparative fungicidal test appear in Table III.

TABLE III

Percent Inhibition

<u>Compound</u>	<u>P</u>	<u>H</u>	<u>E</u>	<u>B</u>
5 3,5-dimethyl-4-chloro-1-hydroxymethylpyrazole	100	85	90	70
3,5-dimethyl-4-bromo-1-hydroxymethylpyrazole	100	90	95	90
3,5-dimethyl-4-iodo-1-hydroxymethylpyrazole	90	75	75	75
10 3,5-dimethyl-4-nitro-1-hydroxymethylpyrazole	90	85	75	85

P = *Pythium ultimum*H = *Helminthosporium sativum*F = *Fusarium oxysporum*R = *Rhizoctonia solani*

EXAMPLE 5

A composition in accordance with the invention comprising a water solution containing 1.5 percent (about 15,000 ppm) on a weight by volume basis of a compound containing 98.0 percent on a weight by weight basis of 1-hydroxymethyl-3,5-dimethylpyrazole was prepared by dissolving 30 grams of the compound (technical grade) in 20 liters of water.

Cauliflower was planted in an 0.04 hectare field following the standard agricultural practice for this vegetable. When the heads were being formed, the stems became infested with *Erwinia caratovora*. This bacterium is the causal agent of soft rot, and produces an enzyme which interacts with the middle lamella of the host tissue. The cells in the older decayed tissue eventually become plasmolyzed. All of these effects result in soft rot.

The cauliflower was sprayed with the aforementioned composition until liquid ran off of the foliage. Three days later, the soft rot wounds were observed to be dried, an indication that the *Erwinia* bacterium was no longer active. The treated plants were observed to be recovered completely. Furthermore, no phytotoxicity was induced on the cauliflower plant by spraying this composition. The degree of infection in the cauliflower by the *Erwinia* bacterium was recorded on the basis of the following key:

	Very severe bacterial infection/abundant soft rot tissue in cauliflower stems	5
	Severe bacterial infection	4
	Moderate bacterial infection	3
5	Slight bacterial infection	2
	Soft rot host-tissue became dried	1
	Absence of bacterial infection/host tissue wounds dried and plants recovered	0

The results of this test are summarized in the following Table IV:

10

TABLE IV

Average Rating of Disease

Incidence After Spraying

<u>Treatment</u>	<u>Dosage</u>	<u>3 days</u>	<u>10 days</u>
Untreated Control	0	5	5
Treated with composition	1.5% solution	1	0

EXAMPLE 6

A composition in accordance with the invention containing 5 percent on a weight by volume basis of the active compound in water as in Example 5 was prepared by dissolving 100 grams of the technical grade of the compound in 20 liters of water.

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This 5 percent composition was then sprayed to run-off on the foliage of cauliflower plants growing in standard producing fields. No phytotoxicity was observed as the result of spraying with this 5 percent solution.

EXAMPLE 7

Two species of orchids were infected with *Pseudomonas* sp. These two orchids were: *Dendrobium* and *Oncidium*. The infected orchid plants were sprayed to foliage run-off with a 1.5 percent composition of the active compound as prepared in Example 5. Readings were taken at 3 and 7 days after spraying. No phytotoxicity was observed as the result of spraying with this 1.5 percent solution. The results obtained were recorded on the basis of the following key:

30

	Very severe bacterial infection	5
	Severe bacterial infection	4
	Moderate bacterial infection	3
	Slight bacterial infection	2
5	Very slight bacterial infection	1
	No bacterial infection	0

The results of this test for both orchid species were equivalent and are summarized in the following Table V. This test may indicate a need to test larger dosages for Pseudomonas sp. control on orchids.

10

TABLE V

<u>Treatment</u>	<u>Dosage</u>	<u>Average Rating of Disease</u>	
		<u>Incidence After Spraying</u>	
		<u>3 days</u>	<u>10 days</u>
Untreated Control	0	4	4
Treated with Composition	1.5% solution	2	3

EXAMPLE 8

A cauliflower field infested with the fungus Peronospora parasitica was sprayed with the 1.5 percent composition as prepared in Example 5. The Peronospora fungus attacks the foliage and cauliflower head producing sunken dark spots (small to larger than one inch), which make the agricultural product unfit for human consumption. Three days after spraying, the infested areas were observed to have died. No phytotoxicity was observed by spraying with the composition.

20

The disease incidence in the cauliflower was recorded on the basis of the following key:

Severe disease incidence	3
Moderate disease incidence	2
Slight disease incidence	1
Disease incidence controlled	0

The following Table VI summarizes the results of this test:

30

TABLE VI

Average Disease Rating

<u>Treatment</u>	<u>Dosage</u>	<u>Before Spraying</u>	<u>3 Days after Spraying</u>
Untreated			
5 Control	0	3	3
Treated with	1.5%		
Composition	solution	3	0

EXAMPLE 9

A composition containing 1-hydroxymethyl-3,5-dimethylpyrazole was tested for controlling fungal and bacterial diseases interfering with cabbage seed germination. The *Aspergillus* sp. and bacteria are known to affect seed germination and vigor. The same amount of seed was soaked for 10 minutes in different treatments. The seed was then sprayed on tissue paper and its germination was observed. Temperature and water conditions were maintained constant for all the treatments in this germination test. The results of this test were recorded on basis of seed germination and seedling vigor based on the following key:

Excellent germination/vigor	3
Good germination/vigor	2
Poor germination/vigor	1
No germination	0

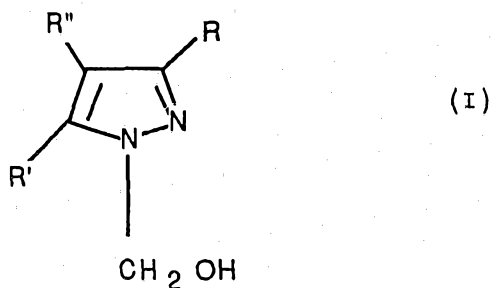
It should be noted that seedling vigor is considered as index of the treatment phytotoxicity, and on this basis, no phytotoxicity was observed. The following Table VII summarizes the results of this test:

TABLE VII

<u>Treatment</u>	<u>Dosage</u>	<u>Soaking Time</u>	<u>Germination Rating</u>	<u>Sprouting Days After Seeding</u>
Untreated Seed	-	0	1	5
Hot Water at 55°C	-	10 mins.	3	2
Treated with				
composition	0.5%	10 mins.	3	5
	solution			

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for controlling microorganisms on a plant comprising applying to said plant or the seed thereof an amount sufficient to inhibit the growth and proliferation of at least one microorganism on said plant or seed of a compound of the formula I



wherein R and R' are independently selected from hydrogen and an alkyl of 1 to 4 carbon atoms, and R'' is a moiety selected from the group consisting of (a) hydrogen, (b) a halogen atom selected from the group consisting of F, Cl, Br and I, and (c) a nitro group, or the hydrochloride salt of said compound.

2. The method of Claim 1, wherein said applying comprises spraying a composition comprising the compound of formula I.
3. The method of Claim 2, wherein said sufficient amount is in the range of about 2,500 to about 30,000 parts per million of said composition.
4. The method of Claim 3, wherein said sufficient amount is in the range of about 15,000 to about 25,000 parts per million of said composition.
5. The method of Claim 1, wherein said compound is 3,5-dimethyl-1-hydroxymethylpyrazole.
6. The method of Claim 1, wherein said compound is 3,5-dimethyl-1-hydroxymethylpyrazole hydrochloride.

7. The method of Claim 1, wherein said microorganism is selected from the group consisting of bacteria and fungi.

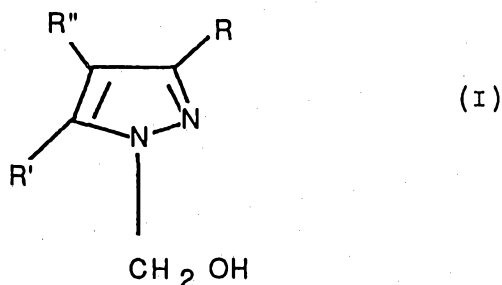
8. The method of Claim 7, wherein said microorganism is a bacteria selected from the group consisting of *Erwinia caratovora*, *agrobacteria*, *corynebacteria*, *Xanthomonas sp.*, *Pseudomonas sp.*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

9. The method of Claim 7, wherein said microorganism is a fungus selected from the group consisting of *Pythium ultimum*, *Helminthosporium sativum*, *Rhizoctonia solani*, *Monilia fructicola*, *Fusarium oxysporum*, *Peronospora parasitica* and *Alternaria solani*.

10. The method of Claim 1, wherein said plant or seed is selected from the group consisting of ornamental plants, fruit plants, vegetable plants, cereal crop plants and seeds thereof.

11. The method of Claim 1, comprising applying said compound or hydrochloride salt to said plant or seed two or more times.

12. A composition for controlling microorganisms on a plant comprising:
 (a) a compound of the formula



wherein R and R' are independently selected from hydrogen and an alkyl of 1 to 4 carbon atoms; and R'' is a moiety selected from the group consisting of (a) hydrogen, (b) a halogen atom selected from the group consisting of F, Cl, Br and I, and (c) a nitro group; or the hydrochloride salt of said compound, in an amount per unit weight of said composition sufficient to inhibit the growth and proliferation of at least one microorganism on said plant or seed thereof where one or more applications of said composition are undertaken; and

(b) an inert carrier.

13. The composition of Claim 12, wherein said compound is 3,5-dimethyl-1-hydroxymethylpyrazole.

14. The composition of Claim 12, wherein said compound is 3,5-dimethyl-1-hydroxymethylpyrazole hydrochloride.

15. The composition of claim 13, further comprising at least one inert additive relative to the compound of formula I and said additive is selected from the group consisting of stabilizers, spreading agents, wetting agents, sticking agents, fillers, bactericides, fungicides, pesticides and any combination thereof.

16. The composition of Claim 13, in the form of a solution or dispersion, wherein said carrier is inert relative to the compound of formula I and is selected from the group consisting of liquids and powders.

17. The composition of Claim 17, wherein said carrier is water.

18. The composition of Claim 17, wherein said carrier is a flowable powder.

DATED THIS 12TH DAY OF JULY, 1991

BUCKMAN LABORATORIES INTERNATIONAL, INC.

WATERMARK PATENT & TRADEMARK ATTORNEYS

THE ATRIUM

290 BURWOOD ROAD

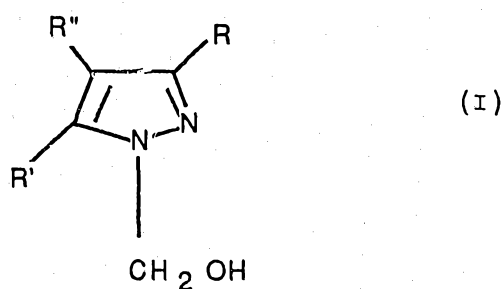
HAWTHORN VICTORIA 3122

AUSTRALIA

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ABSTRACT

A method of protecting plants and seeds thereof which are susceptible to attack by microorganisms. To the plant or seed is applied a compound having the formula



wherein R and R' are independently selected from hydrogen and alkyl of 1 to 4 carbon atoms; and R'' is a moiety selected from the group consisting of (a) hydrogen, (b) a halogen atom selected from the group consisting of F, Cl, Br and I, and (c) a nitro group or the hydrochloride salt of the compound. The method disclosed is especially useful for protecting ornamental plants, vegetable plants and cereal crop plants and seeds thereof which are susceptible to attack by microorganisms. A composition containing the compound of formula I for use in protecting plants which are susceptible to attack by microorganisms is also provided.