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(54) Title: MATERIALS AND METHODS FOR THE CONTROL OF BACTERIAL WILT IN PLANTS

(57) Abstract: The subject invention concerns compositions and methods useful for the control of plant pathogens. Specifically exemplified are extracts from Yucca plants which are effective in the control of bacterial wilt in plants. In an exemplified embodiment, an extract from *Yucca schidigera* is used to control bacterial wilt in a plant caused by *Ralstonia solanacearum*.



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DESCRIPTION

MATERIALS AND METHODS FOR THE CONTROL OF BACTERIAL WILT IN
PLANTS

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CROSS-REFERENCE TO A RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application Serial No. 60/636,470, filed December 16, 2004, which is hereby incorporated by reference herein in its
10 entirety, including any figures, tables, and drawings.

BACKGROUND OF THE INVENTION

Plants are subject to attack by a great number of pathogens. These pathogens can be, for example, bacteria, fungi, or nematodes. Pesticidal compounds have long been used to
15 increase yields and extend agricultural production capabilities into new areas. They have also been extremely important tools for ameliorating season-to-season differences in yield and quality caused by weather-driven variations in disease pressure.

The future role of pesticides in agriculture is increasingly threatened by several factors including the development of pest resistance, increasing concerns about food safety,
20 and environmental accumulation of toxic compounds. As older pesticides are removed from the market due to regulatory changes, and new pesticides are becoming increasingly expensive to register, there is an increasing need to find ways to more wisely use the remaining, safest pesticides. This is particularly true for the many crop/disease combinations which do not represent large enough markets to pay for the cost of new compound
25 registration. Wiser pesticide use will include ways to reduce application rates (and thus potential residues), finding ways to extend registrations to new crops, and identifying new compositions and treatments to combat the development of pest resistance.

Chemical pesticides have provided an effective method of control; however, the public has become concerned about the amount of residual chemicals which might be found
30 in food, ground water and the environment. Stringent new restrictions on the use of chemicals and the elimination of some effective pesticides from the market place could limit economical and effective options for controlling pests. In addition, the regular use of chemical toxins to control unwanted organisms can select for resistant strains.

The value of vegetable crops grown in the state of Florida totaled over \$1.79 billion for the 1991-92 crop season with much of the cabbage, cucumber, pepper, and tomato crops planted as transplants. Tomato alone accounts for \$735 million in this production total and over 80% of the crop is transplanted. The use of vegetable transplants in commercial field production systems is important in many areas of the United States. In California, all of the celery, fresh market tomato and pepper, and most of the cauliflower and broccoli are grown as transplant crops. In Monterey County alone, the value of the transplants grown in 1992 amounted to nearly \$18 million and represented a final crop value of \$176 million.

Currently, the most effective substance for soil treatment of pathogens is methyl bromide. Methyl bromide is used in the control of pest insects, nematodes, weeds, pathogens, and rodents. In the United States, about 27,000 tons of methyl bromide is used annually in agriculture, primarily for soil fumigation, as well as for commodity and quarantine treatment, and structural fumigation. Globally, about 76,000 tons of methyl bromide are used each year.

When used as a soil treatment, methyl bromide is injected into the soil at a depth of 12 to 24 inches before a crop is planted. This will effectively sterilize the soil, killing the vast majority of soil organisms. Immediately after the methyl bromide is injected, the soil is covered with plastic tarps that hold most of the methyl bromide in the soil. The tarps are removed 24 to 72 hours later. After the tarps are removed, much of the methyl bromide leaves the soil. The EPA estimates that about 50% to 95% of the methyl bromide in the soil eventually enters the atmosphere.

While methyl bromide in large doses can result in damage to the human nervous system and respiratory system, the greatest danger posed by methyl bromides is the damage to the ozone layer. According to the 1994 Assessment of Ozone Depletion, the Ozone Depletion Potential (ODP) of methyl bromide has been assessed to be 0.6. This makes the ODP of the methyl bromide fifty times more effective at destroying ozone than CFC's on a per molecule basis.

According to the Clean Air Act (1990 Amendments), all substances with an ODP of 0.2 or greater are to be phased out in the United States. This means that methyl bromide will need to be phased out. There has been legislation to ultimately prohibit the production and importation of methyl bromide in the United States. In addition, 160 countries have signed the Montreal Protocol, a treaty calling for the levels of ozone-depleting chemicals to be

frozen at 1991 levels. In addition, the EPA is lobbying for nations to stop using methyl bromide all together.

In light of the environmental problems with methyl bromide, and the continuing need for a soil treatment, an environmentally safe chemical alternative has been sought. Thus, there remains a need for pathogen control methods which are more compatible with the need for affordable and effective disease control, a high degree of food safety, and minimal environmental impact.

Ralstonia solanacearum (Smith) (Rs) Yabuuchi *et al.* (1995), causal agent of bacterial wilt, is a pathogen of serious economic concern in the southeastern United States, as it is throughout the tropical and sub-tropical regions of the world. It is very destructive to many crops infecting over 450 plant species, including many economically important crops including tomato, potato, and banana. Brown rot of potato caused by Rs race 3/biovar 2 (r3/b2) has been estimated to affect about 3.75 million acres in 80 countries (except US and Canada) with global economic loss estimates currently more than \$950 million per year (DEFRA, 2003). Bacterial wilt caused by *Ralstonia solanacearum* (Rs) is a major disease problem in fresh tomato production fields in north Florida.

Strains of *R. solanacearum* are grouped into five races according to the host or hosts primarily affected and five biovars according to the use of selected biochemical properties (Hayward, 1991). Rs r3/b2, which is adapted to temperate climates, has been responsible for recent outbreaks of potato brown rot disease in Western Europe (Elphinstone *et al.*, 1996; Janse, 1996) and other regions (Hayward, 1991) of the world as well as on geraniums (*Pelargonium*) in several states in the United States (Williamson *et al.*, 2002). Rs r3/b2 has a host range primarily limited to potato, tomato, geranium, common solanaceous and, in favorable conditions, on non-solanaceous weeds (Elphinstone *et al.*, 1996; Janse, 1996; Tusiime *et al.*, 1998).

Latent infections in seed potato tubers and geranium cuttings have lead to the spread of this biovar internationally. The Rs r3/b2 strains are adapted to cooler temperature and would be particularly damaging to potato production regions of the United States (New Pest Response Guidelines, *Ralstonia solanacearum* race 3/biovar 2, Southern Wilt of Geranium, USDA, January 16, 2004, Version 4.0) and might affect tomato production and other host plants in expanded geographic and climatic ranges in the United States (Momol, 2003). Control of brown rot is dependent on detection of the bacterium at this latent stage. A

detection assay is required which is sensitive while being rapid and specific. Serological techniques including immunofluorescence (IF) and enzyme-linked immunosorbent assay (ELISA) and molecular techniques such as PCR have been used for detecting *Rs*.

Rs is mainly spread through latently infected plant materials as well as through infested irrigation water and soil. It survives in surface water and soil usually by establishing itself in host plants. Due to limited efficacy of current integrated management strategies, bacterial wilt continues to be economically important for many economically important crops in the United States, and many subtropical, tropical, and temperate areas of the world. Cultural practices, crop rotation and host resistance may provide limited control (Pradhanang *et al.*, 2003).

Since 1995, *Rs* r3/b2 has entered several states of the United States on various occasions in commercially grown greenhouse geraniums. The pathogen was traced back to infected geraniums cuttings that were imported from Guatemala (Williamson *et al.*, 2002; Kim *et al.*, 2003). R3/b2 of *Rs* is a listed "select agent" in the United States under the Agricultural Bioterrorism Protection Act of 2002 (Federal Register, December 13, 2002, Part V, Department of Agriculture, APHIS, 7 CFR Part 331, 9 CFR Part 121) because it has the potential to be a severe threat to the potato industry and other crops and does not established in the United States. In February 2003, the USDA/APHIS confirmed the presence of *Rs* r3/b2 in geraniums in four States. "As of May 21, 2003, APHIS confirmed detection of *Rs* r3/b2 on geraniums in 27 States and 127 establishments (excluding the MI and NH rooting stations), primarily in geraniums from Kenya. The number of positive testing facilities in each state is tallied as follows: AL (9), AR (2), CO (1), DE (1), GA (2), ID (1), IL (4), IN (4), IA (6), KS (3), KY (1), MD (3), ME (1), MI (13), MN (4), MO (6), NH (1), NJ (1), NY (3), NC (13), OH (7), PA (8), SC (8), TN (1), TX (7), VA (10), and WI (7). All of the 921 affected nurseries have been released. APHIS is in the process of following up on suspect geraniums linked to a few shipments from Guatemala that tested positive" (Update from USDA, APHIS, PPQ, May 28, 2003). In December 2003 and January 2004, *Rs* r3/b2 was detected again from geranium cuttings imported to the United States from Guatemala (O'Hern, 2004).

BRIEF SUMMARY OF THE INVENTION

The subject invention concerns compositions comprising an extract from *Yucca* plant and methods of use to control pathogens in plants, such as those that cause bacterial wilt

disease. In one embodiment, the bacterial wilt is caused by *Ralstonia solanacearum*. In an exemplified embodiment, an extract used in the compositions of the invention is obtained from the Yucca plant *Yucca schidigera*. The subject invention also concerns methods for preventing, controlling, or minimizing bacterial wilt in plants. In one embodiment, a composition comprising an extract of Yucca of the present invention is applied to the plant itself or to soil in which the plant is planted.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows incidence of bacterial wilt on tomato growing in soil with or without Yucca extract treatment under greenhouse conditions. UTC = untreated control.

Figure 2 (photograph) shows the effect of Yucca extract on bacterial wilt incidence under greenhouse conditions. Photo was taken 25 days after tomato transplanting.

Figures 3A-3C show the effect of different products on bacterial wilt incidence. Figure 3A: STBX-016 (Phyton 27) (Source Tech Bio, Inc., Edina, MN); Figure 3B: Yucca extract; Figure 3C: QRD 600 (Muscodor) (AgraQuest Inc., Davis, CA). Photograph was taken 30 days after transplanting.

DETAILED DISCLOSURE OF THE INVENTION

The subject invention concerns materials and methods for the effective and environmentally safe control of plant pathogens. A composition of the invention comprises an extract from a Yucca plant to control plant pathogens that cause bacterial wilt, brown rot, and other diseases in plants. Yucca plants contemplated within the scope of the invention include, but are not limited, to *Yucca schidigera*, *Yucca valida*, *Yucca whipplei*, and *Yucca elata*. In one embodiment, the bacterial wilt is caused by *Ralstonia solanacearum*. The Rs can be race 1, race 2, race 3, race 4, or race 5. In a specific embodiment, the bacteria is *Ralstonia solanacearum* race 1 biovar 1. In an exemplified embodiment, a composition of the present invention comprises an extract obtained from the Yucca plant *Yucca schidigera*. Extracts of *Yucca schidigera* are commercially available (Agroindustries El Alamo, Ensenada, Baja California, Mexico; Wescotek, Terminal Island, CA, USA) and can be used in the present invention. Extract from Yucca plants can be prepared by a variety of methods known in the art including, for example, cold press extraction, solid phase extraction, microwave-assisted extraction, surfactant medicated extraction, and supercritical fluid

extraction (see, for example, Huie, 2002; U.S. Patent Nos. 6,733,798 and 6,646,144; and published U.S. patent application No. 20030005484).

In one embodiment, a composition of the invention can comprise from about 0.01% (v/v) to about 10% (v/v) extract of Yucca in a suitable solvent, such as water. In another embodiment, a composition of the invention comprises 0.1% to 5%, and more preferably about 1% extract of Yucca in a solvent. In an exemplified embodiment, a composition comprises about 1% of Yucca extract from *Yucca schidigera* in water. Compositions of the invention comprising Yucca extract can also comprise alcohol, such as ethanol, and/or detergents. Alcohol concentrations can be from about 0.1% to about 5% and detergent concentrations can be from about 0.01% to about 5%. In one embodiment, a composition comprises about 1% to about 2% ethanol and/or about 0.1% to about 0.5% detergent in a suitable solvent. Compositions of the invention can also comprise other pathogen control agents, such as bactericides and/or fungicides, *etc.* The subject invention also concerns extract of a Yucca plant in a formulation suitable for a dipping or spraying application.

The subject invention also concerns methods for controlling plant pathogens. In one embodiment, a method is provided for preventing, controlling, minimizing, or treating bacterial wilt disease in plants. In one embodiment, an extract of Yucca of the present invention is applied to the plant itself and/or to soil in which the plant will be or is planted. For example, the plant can be sprayed with the extract composition or the plant can be dipped into the extract composition. In one embodiment, the bacterial wilt is caused by *Ralstonia solanacearum*. The Rs can be race 1, race 2, race 3, race 4, or race 5. In a specific embodiment, the bacteria is *Ralstonia solanacearum* race 1 biovar 1. In one embodiment, a method of the invention further comprises treating a plant and/or soil with a bactericide, a fungicide, a chemical control agent, and/or a biological control agent. In a specific embodiment, the method comprises treating a plant and/or soil with an extract of Yucca of the present invention, and also treating the plant or soil with a composition comprising ACTIGARD (Syngenta Crop Protection, Greensboro, NC) (acibenzolar-S-methyl). The bactericide, fungicide, chemical and biological control agents can be applied to the plant and/or soil prior to, at the same time or in combination with, or subsequent to the application of the composition comprising the extract of Yucca plant. In one embodiment, compositions comprising the Yucca extract of the invention can be applied 2-3 weeks before planting to soil or growing media as a drench, through drip irrigation or other methods. Foliar application can be made, for example, every 7-10 days during growing season.

Advantageously, compositions of the subject invention are environmentally safe. Active ingredients in the compositions comprise plant extracts or are derived from plant extracts. In one embodiment, a composition of the invention may comprise an individual bactericide and/or fungicide and/or a surfactant that acts as a wetting agent, such as a natural or synthetic detergent. The wetting agent helps during the dilution of the chemical compositions in water. The wetting agent also helps the chemical composition saturate the soil when soil is being treated. Preferably, a food grade surfactant is used due to the use of the compositions as soil or plant treatments. An example of a suitable surfactant is polysorbate. The amount of surfactant can be readily determined by a person skilled in the art and should be an amount sufficient to set fully the treated soil when the first dosage of a composition of the present invention is applied. This amount may change with soil composition and crop type. The balance of the composition can be completed with oil and water, and other compounds as appropriate.

The compositions and methods described herein can be used to control a broad range of plant pathogenic bacteria and fungi. These targets include, but are not limited to, species of *Ralstonia* sp. and *Pseudomonas* sp.

Using the subject invention, plant pathogens can be controlled in the soil and on seeds, corms, bulbs, flowers, stems, leaves, exposed roots, and fruits of plants including, but not limited to, grapes, pears, apples, peaches, nectarines, grapefruit, cherries, apricots, lemons, limes, oranges, mangos, bananas, pineapple, and tangerines. Any plant which is susceptible to bacterial wilt disease caused by Rs is encompassed within the scope of the methods of the present invention. The plants which may be treated further include, but are not limited to, tomatoes, potatoes, bananas, peas, alfalfa, cabbage, clover, kale, lentil, soybean, sweet potato, radish, grape, cotton, sunflower, rape, chicory, chickpea, sorghum, onion, coconut, lily, sugarcane, cucumbers, squash, zucchini, eggplant, chili pepper, bell pepper, tobacco, groundnut, lettuce, cantaloupes, ginger, rice, corn, wheat, oats, barley, rye, millet, and other cereals, turfgrasses, and flower crops, including geraniums, other ornamental plants, nightshades, and almonds.

Compositions of the subject invention comprising Yucca extract can be used to control microbial plant disease on both dormant and non-dormant plant tissue. As known by those skilled in the art, non-dormant tissue includes growing vegetation and fruits (pre- and post-harvest).

In cases where compositions of the invention are useful for eradication of existing infections of fruit or plants, the further protection of that fruit or plant from subsequent infections can be achieved by the simultaneous or subsequent application of a fungicide, bactericide, or a biological control organism in a dip or spray application. This application
5 can also be made along with the application of various waxes or finishes which are commonly used with fruit. The formulation of such applications can also include nutrients which will benefit the establishment of the biocontrol organism. Appropriate formulations and concentrations can be readily ascertained by those skilled in the art using the teachings of the subject invention.

10 The subject invention also concerns compositions comprising extract of a Yucca plant and one or more fungicides and/or one or more bactericides and the use of the compositions to treat soil and/or plants that are infected with one or more pathogens or that may be susceptible to infection by one or more pathogens. The potent activity of the plant extracts of the subject invention combined with other fungicides and/or bactericides makes it possible to
15 achieve the same level of pathogen control while using a smaller quantity of the extract component or a smaller quantity of the fungicide and/or bactericide. Compositions of the present invention can comprise a mixture of components wherein the mixture is sufficiently active so that application of the composition enables utilization of reduced amounts of each of the active ingredients while still providing effective activity. This is significant because
20 lower use rates lead to lower residues on the crop or in the environment, lower costs of application, an expansion of the margin between crop safety and efficacy for bactericides and fungicides which can be phytotoxic (thus enhancing their safety or expanding the crops, varieties or timings for their use), and lower total "market basket" exposure for a multi-use fungicide or bactericide. Combinations of other fungicides or bactericides with a plant
25 extract of the invention offer additional advantages because of the particular mode of action of these materials. In one embodiment, a composition of the invention comprises extract of Yucca and acibenzolar-S-methyl.

Chemical control agents which can be combined with or used with a plant extract of the subject invention include, but are not limited to, benomyl; borax; captafol; captan;
30 chlorothalonil; various formulations containing copper; various formulations containing zinc; dichlone; dicloran; iodine; various ergosterol biosynthesis inhibiting fungicides including but not limited to fenarimol, imazalil, myclobutanil, propiconazole, prochloraz, terbutrazole, flusilazole, triadimefon, and tebuconazole; folpet; iprodione; mancozeb; maneb; metalaxyl;

oxycarboxin; oxytetracycline; PCNB; pentachlorophenol; quinomethionate; sodium arsenite; sodium DNOC; sodium hypochlorite; sodium phenylphenate; streptomycin; sulfur; thiabendazole; thiophanate-methyl; triforine; vinclozolin; zineb; ziram; tricyclazole; cymoxanil; blasticidin; and validimycin. A plant extract of the invention can also be
5 combined with various spray oils.

Biological control agents that can be combined with or used in conjunction with a plant extract of the subject invention include, but are not limited, to *Bacillus* sp., *Trichoderma* sp., *Erwinia* sp., *Pichia* sp., *Candida* sp., *Cryptococcus* sp., *Talaromyces* sp., *P. fumosoreus*, *B. bassiana*, *Chaetomium* sp., *Gliocladium* sp., *Aureobasidium* sp., *Dabaryomyces* sp.,
10 *Exophilia* sp., *Ampelomyces* sp., and *Mariannaea* sp.

The present invention also encompasses several methods for the treatment of soil. In one embodiment, the method involves diluting a plant extract of the invention in water or another suitable solvent. In one embodiment, a Yucca plant extract is diluted to about 1% in water. The composition used for treating soil can also comprise a detergent and/or alcohol as
15 indicated herein. The diluted solution is then sprayed onto or into the ground. In one embodiment, the diluted solution is applied to a depth of 12 to 24 inches into the soil. Preferably, the amount sprayed is the quantity necessary to saturate the soil. This amount is between typically about 1% to 5% by weight of the soil being treated. The amount depends on the type of soil being treated and the crop being grown. The subject invention also
20 concerns soil that has been treated using a composition of the invention.

A further aspect of the subject invention pertains to containers in which the compositions of the subject invention are sold and/or distributed. In a preferred embodiment, these containers contain a composition comprising a plant extract of the invention and have instructions for the use of the extract for the control of plant pathogens. In one embodiment,
25 the containers are plastic (or other appropriate inert material). The formulation in the container is preferably provided concentrated but may also be provided in diluted form for immediate use. In a preferred embodiment, the instructions pertain to the use of a plant extract as fumigants for the control of plant pests. In a further preferred embodiment, the instructions for use are written on the outside of the container.

30 All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

5 EXAMPLE 1—OTHER FORMULATIONS

A. Wettable powders. Wettable powders are water-dispersible compositions containing the active material, an inert solid extender, and one or more surfactants to provide rapid wetting.

10 The inert extenders which are preferred for use in the wettable powders of this invention containing the active compounds are of mineral or organic origin. Extenders suitable for the wettable powder formulations of this invention are the natural clays, vermiculite, diatomaceous earth, and synthetic mineral fillers derived from silica and silicate. Most preferred filters for this invention are kaolinites, attapulgite clay, montmorillonite clays, synthetic silicas, synthetic magnesium silicate, and calcium sulfate
15 dihydrate. A surface active agent can also be added to give a homogenous and stable formulation.

Among the more preferred surfactants are the nonionic and anionic types. They are most suitable for the preparation of dry, wettable products of this invention and dispersants. Occasionally a liquid, non-ionic compound which is primarily an emulsifier may serve as
20 both wetter and dispersant.

Most preferred wetting agents are alkylbenzene and alkylnaphthalene sulfonates, sulfated fatty alcohols, amines, or acid amides, long chain esters of sodium isethionate, esters of sodium sulfosuccinate, sulfated or sulfonated vegetable oils, and ditertiary acetylenic glycols. Preferred dispersants are methyl cellulose, polyvinyl alcohol, lignin sulfonates,
25 polymeric alkylnaphthalene sulfonates, sodium naphthalene sulfonates, polymethylene bisnaphthalene sulfonate, and sodium-N-methyl-N-(long chain acid) taruates.

Wetting and dispersing agents in these preferred wettable powder compositions of the invention are usually present at concentrations of from about 0.5 weight percent to 5 weight percent. The inert extender then completes the formulation. Where needed, 0.1 weight
30 percent of the extender may be replaced by a corrosion inhibitor or an anti-foaming agent or both.

Thus, wettable powder contains a corrosion inhibitor or an anti-foaming agent or both, the corrosion inhibitor should not exceed about 1 percent of the composition, and the anti-

foaming agent should not exceed about 0.5 percent by weight of the composition, both replacing equivalent amounts of the inert extender.

B. Dusts. Dusts are dense powder compositions which are intended for application in dry form. Dusts are characterized by their free-flowing and rapid settling properties so that they are not readily windborne to areas where their presence is not desired. They contain primarily an active ingredient and a dense, free-flowing, solid extender. Their performance is sometimes aided by the inclusion of a wetting agent and convenience in manufacture frequently demands the inclusion of an inert absorptive grinding aid.

The wettable powder as described above can also be used in the preparation of dusts. While such wettable powders can be used directly in dust form, it is more advantageous to dilute them by blending with the dense dust diluent. In this manner, dispersing agents, corrosion inhibitors, and anti-foam agents may also be used as components of a dust.

Thus, the dust compositions of this invention can comprise from about 0.5 to 20.0 weight percent active ingredient, 5 to 25 weight percent filler, 0.0 to 1.0 weight percent wetting agent, and from about 30 to 90 weight percent dense, free-flowing extender, as these terms are used herein. Such dust formulations can contain, in addition, minor amounts of dispersants, corrosion inhibitors, and anti-foam agents derived from the wettable powders used to make the dust.

C. Emulsifiable oils. Emulsifiable oils are usually solutions or suspensions of active material in non-water miscible solvents together with a surfactant and/or emulsifier.

For compositions of this invention, emulsifiable oil compositions can be made by mixing the active ingredient with an organic solvent and surfactant. Suitable solvents for the compositions of this invention are chlorinated solvents, water immiscible ethers, esters, or ketones alone or in admixture with aromatic hydrocarbons. Suitable surfactants are those ionic or non-ionic agents known to the art as emulsifying agents.

Emulsifying agents most suitable for the emulsifiable oil compositions of this invention are long chain alkyl or mercaptan polyethoxy alcohols, alkylaryl polyethoxy alcohols, sorbitan fatty acid esters, polyoxyethylene ethers with sorbitan fatty acid esters, polyethylene glycol esters with fatty rosin acids, fatty alkylol amide condensates, calcium and amine salts of fatty alcohol sulfates, oil soluble petroleum sulfonates, or preferably mixtures of these emulsifying agents should comprise from about 1 to 10 weight percent of the total composition. As described above, however, up to 5 parts of emulsifying agent for each part of active ingredient can be used.

Thus, emulsifiable oil compositions of the present invention can consist of from about 10 to 50 weight percent active ingredient, about 40 to 82 percent solvents, and about 1 to 10 weight percent emulsifier, as these terms are defined and used above.

D. Granules. Granules are physically stable, particulate compositions containing
5 spores and/or mycelia of this invention which adhere to or are distributed through a basic matrix of a coherent, inert carrier with microscopic dimensions. In order to aid leaching of the active ingredient from the granule, a surfactant can be present.

The inert carrier is preferably of mineral origin, and suitable carriers are natural clays, some pyrophyllites and vermiculite. Suitable wetting agents can be anionic or non-ionic.

10 For the granule compositions of this invention, most suitable carriers are to two types. The first are porous, absorptive pre-formed granules, such as preformed and screened granular attapulgite or heat expanded, granular, screened vermiculite. On either of these, a solution of the active agent can be sprayed and will be absorbed at concentrations up to 25 weight percent of the total weight. The second type are initially powdered kaolin clays,
15 hydrated attapulgite, or bentonite clays in the form of sodium calcium, or magnesium bentonites. Water-soluble salts such as sodium salts may also be present to aid in the disintegrations of the granules in the presence of moisture. These ingredients are blended with the active component distributed uniformly throughout the mass. Such granules can also be made with 25 to 30 weight percent active component but more frequently a concentration
20 of about 10 weight percent is desired for optimum distribution. The granular compositions of this invention are believed to be most useful in a size range of 15-30 mesh.

The most suitable wetting agents for the granular compositions of this invention depend upon the type of granule used. When pre-formed granules are sprayed with active material in liquid form, the most suitable wetting agents are non-ionic, liquid wetters miscible
25 with the solvent. These are more generally known in the art as emulsifiers and comprise alkylaryl polyether alcohols, alkyl polyether alcohols, polyoxethylene sorbitan fatty acid esters, polyethylene glycol esters with fatty or rosin acids, fatty alkylol amide condensates, oil petroleum or vegetable oil sulfonates, or mixtures of these. Such agents will usually comprise up to about 5 weight percent of the total composition.

30 When the active ingredient is first mixed with a powdered carrier and subsequently granulated, liquid non-ionic wetters can still be used, but it is usually preferable to incorporate at the mixing stage one of the solid, powdered anionic wetting agents such as

those previously listed for the wettable powders. Such agents should comprise about 0 to 2 percent of the total composition.

Thus, the preferred granular formulation of this invention comprises about 5 to 30 weight percent active material, about 0 to 5 weight percent wetting agent, and about 65 to 95 percent inert mineral carrier, as these terms are used herein.

EXAMPLE 2—ACTIVITY OF YUCCA EXTRACT

Yucca extract (Agroindustries El Alamo) at a concentration of 1% was found effective to eliminate *Ralstonia solanacearum* (Rs) in water when Yucca extract was added to water containing Rs (10^5 cells per ml) and the mixture was incubated with shaking at room temperature for 5 hours.

Yucca extract also significantly reduced bacterial wilt incidence on tomatoes in a greenhouse experiment (Figures 1 and 2). Yucca extract, at a rate of 1 ml per 100 ml of soil, was added to soil infested with Rs at a concentration of 10^8 cells per gram of soil. After a 4-day incubation in a greenhouse, tomato seedlings of a susceptible cultivar Solar Set were transplanted in “Cone-tainers” containing the Yucca extract-treated soil. Tomato plants transplanted in infested soil without treatment with Yucca extract were used as a control. Tomato plants were kept in a greenhouse and bacterial wilt incidence was recorded weekly and quantified as percentage of plants wilted. All plants growing in the soil without Yucca extract treatment wilted in two weeks after transplanting (inoculation), while only 25% of plants wilted in four weeks in Yucca extract-treated soil (Figures 1 and 2).

EXAMPLE 3—EFFICACY OF SEVERAL BIORATIONAL COMPOUNDS FOR CONTROL OF BACTERIAL WILT OF TOMATO UNDER GREENHOUSE CONDITIONS

The efficacy of several compounds was evaluated for control of bacterial wilt of tomato caused by *Ralstonia solanacearum* under greenhouse conditions. STBX-016 (Phyton 27) (Source Tech Bio, Inc., Edina, MN) was applied as foliar spray and soil drench at a concentration of 1ml/liter starting 15 days before tomato seedling transplanting and once every seven days thereafter until four weeks after transplanting. For soil drench, 5 ml of STBX-16 was applied to each transplant cell. Soils used for transplanting were infested with *Ralstonia solanacearum* strain Rs 5 (race 1, biovar 1) at an initial density of 4.2×10^7 CFU/ml of soil. The soils were then treated with STBX-016 at a rate of 2 ml/liter of soil two

hours after soil infestation with the pathogen. In separate treatments, Yucca extracts or QRD 600 (active ingredient is *Muscodor albus*, an endophytic fungus originally isolated from *Cinnamomum zeylanicum*; *M. albus* produces a mixture of volatile organic compounds (VOCs) in culture and has a broad spectrum of antimicrobial activity) (AgraQuest, Inc.) was used to treat the infested soil as STBX-016 at a rate of 10 ml or 7.5 g per liter of soil, respectively. Soils were treated with these products for four days in closed plastic bags and six-week old tomato seedlings were transplanted into treated soils three days later. ACTIGARD (acibenzolar-S-methyl (ISO common name)) (Syngenta Crop Protection, Greensboro, NC) was applied as foliar spray and soil drench. The initial foliar application of ACTIGARD was performed two weeks after seedling emergence at a concentration of 50 µg/ml, followed by a foliar and soil drench application 5 days prior to plant inoculation with the pathogen. For soil drench, 5 ml of ACTIGARD solution at a concentration of 25 µg/ml was applied to each transplant cell. ACTIGARD was sprayed to tomato foliage once every week after transplanting at a concentration of 50 µg/ml. After transplanting the plants were incubated in the greenhouse and arranged as a randomized complete block design with 4 replications. Disease incidence was recorded weekly after transplanting and quantified as the percentage of plants wilted. The data were analyzed using the ANOVA or GLM procedures of the Statistical Analysis System (SAS).

The results indicated that application of STBX-16 or Yucca extract consistently provided significant protection of tomato plants against bacterial wilt. Disease incidence of treated plants was 20 and 25% four weeks after transplanting with STBX-16 and Yucca extract treatments, respectively, while 100% of untreated plants wilted in three weeks (see Table 1 and Figure 3). QRD 600 reduced disease incidence significantly compared to the untreated control, however, it was less effective compared with Yucca extract or STBX-16. Application of STBX-16 or Yucca extract in conjunction with ACTIGARD significantly enhanced disease suppression and combined use of QRD 600 and ACTIGARD showed a tendency to increase disease reduction (Table 1). Thus, these compounds exhibited the capacity to significantly reduce bacterial wilt on the susceptible tomato cultivar used in this study.

Table 1. Effect of Yucca extract and other compounds on incidence of bacterial wilt of tomato under greenhouse conditions.

Treatment ^a	Disease incidence (% plants wilted) ^b			
	Day 7	Day 14	Day 21	Day 28
Untreated	40 a	95 a	100 a	100 a
ACTIGARD	25 b	90 a	95 a	95 a
STBX-016 (Phyton 27)	5 c	5 cd	20 cd	20 cd
STBX-016 and ACTIGARD	0 c	0 d	10 d	10 d
Yucca extract	5 c	15 cd	25 c	25 c
Yucca extract and ACTIGARD	0 c	5 cd	10 d	10 d
QRD 600 (Muscodor)	10 c	30 b	45 b	45 b
QRD 600 and ACTIGARD	10 c	20 bc	30 bc	30 bc

^aSTBX-016 and ACTIGARD were used as foliar spray and/or soil treatment, Yucca extract and QRD 600 were used for soil treatment (see descriptions in the text for timing and dosage of applications).

^bEach treatment had 4 replications with 5 plants in each replication. Values are the means of five replications (20 plants). Same letter in each column indicates no significant difference based on Duncan's multiple range test at $P \leq 0.05$. Day refers number of days after transplanting.

EXAMPLE 4—EFFECT OF YUCCA EXTRACT ON *RALSTONIA SOLANACEARUM* IN WATER

Table 2.

Treatment *	Population before treatment (CFU/ml)	Population 5 hours after treatment (CFU/ml)
Control	2.3×10^5	1.8×10^5
Yucca extract	2.1×10^5	0

* *Ralstonia solanacearum* cells were suspended in 50 ml of sterile deionized water in 250-ml flasks and Yucca extract was used at a final concentration of 1%. The flasks were kept at room temperature with shaking at 200 rpm. Data presented are means of duplicate cultures.

EXAMPLE 5—FORMULATION

Specific formulations which can be used according to the subject invention for field application include, but are not limited to, the following:

0.2 ml Yucca extract, 0.2 ml 95% Ethyl alcohol, 0.1 ml detergent, 19.5 ml water (total
5 volume of 20 ml).

A person skilled in the art, having the benefit of the instant disclosure could readily prepare various volumes of the composition described herein.

10 It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations
15 (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

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CLAIMS

We claim:

- 5 1. A method for controlling a plant pathogen on a plant or in soil, wherein said method comprises contacting said pathogen with a pesticidally effective amount of a composition comprising an extract from a Yucca plant.
2. The method according to claim 1, wherein said composition comprises from about
10 0.01% to about 10% extract of Yucca.
3. The method according to claim 1, wherein said composition comprises from about
 0.1% to about 5% of extract of Yucca.
- 15 4. The method according to claim 1, wherein said composition comprises about 1%
 extract of Yucca.
5. The method according to any of claims 1 to 4, wherein said Yucca plant is *Yucca*
 schidigera.
20
6. The method according to any of claims 1 to 5, wherein said composition comprises an
 alcohol.
7. The method according to claim 6, wherein said alcohol is ethanol.
25
8. The method according to claim 7, wherein said composition comprises from about
 0.1% to about 5% of ethanol.
9. The method according to any of claims 1 to 8, wherein said composition comprises a
30 detergent.

10. The method according to claim 9, wherein said composition comprises from about 0.01% to about 5% of detergent.

5 11. The method according to claim 1, wherein said composition comprises about 1% of extract of *Yucca*, about 1% ethanol, and about 0.05% detergent.

12. The method according to any of claims 1 to 11, wherein said plant pathogen causes bacterial wilt disease in plants.

10

13. The method according to any of claims 1 to 11, wherein said plant pathogen is *Ralstonia solanacearum*.

14. The method according to claim 13, wherein said *Ralstonia solanacearum* is race 1,
15 race 2, race 3, race 4, or race 5 of *Ralstonia*.

15. The method according to claim 13, wherein said *Ralstonia solanacearum* is race 1, biovar 1.

20 16. The method according to any of claims 1 to 11, wherein said composition further comprises a bactericide or a fungicide.

17. The method according to claim 1, wherein said plant is selected from the group consisting of tomatoes, potatoes, bananas, peas, alfalfa, cabbage, clover, kale, lentil, soybean,
25 sweet potato, radish, grape, cotton, sunflower, rape, chicory, chickpea, sorghum, onion, coconut, lily, sugarcane, cucumbers, squash, zucchini, eggplant, chili pepper, bell pepper, tobacco, groundnut, lettuce, cantaloupes, ginger, rice, corn, wheat, oats, barley, rye, and millet.

18. The method according to claim 1, wherein said plant is a flower crop or a turf grass,
30 or an ornamental plant, or a night shade, or an almond plant.

19. The method according to any of claims 1 to 11, wherein said composition further comprises a chemical control agent or a biological control agent.

5 20. The method according to claim 19, wherein said chemical control agent is selected from the group consisting of benomyl, borax, captafol, captan, chlorothalonil, various formulations containing copper; various formulations containing zinc; dichlone, dicloran, iodine, various ergosterol biosynthesis inhibiting fungicides including but not limited to fenarimol, imazalil, myclobutanil, propiconazole, prochloraz, terbutrazole, flusilazole, triadimefon, and
10 tebuconazole; folpet, iprodione, mancozeb, maneb, metalaxyl, oxycarboxin, oxytetracycline, PCNB, pentachlorophenol, quinomethionate, sodium arsenite, sodium DNOC, sodium hypochlorite, sodium phenylphenate, streptomycin, sulfur, thiabendazole, thiophanate-methyl, triforine, vinclozolin, zineb, ziram, tricyclazole, cymoxanil, blasticidin, and validimycin.

15 21. The method according to claim 19, wherein said biological control agent is selected from the group consisting of *Bacillus* sp., *Trichoderma* sp., *Erwinia* sp., *Pichia* sp., *Candida* sp., *Cryptococcus* sp., *Talaromyces* sp., *P. fumosoreus*, *B. bassiana*, *Chaetomium* sp., *Gliocladium* sp., *Aureobasidium* sp., *Dabaryomyces* sp., *Exophilia* sp., *Ampelomyces* sp., and *Mariannaea* sp.

20 22. The method according to any of claims 1 to 11, wherein said extract of *Yucca* is provided in a solvent.

23. The method according to claim 22, wherein said solvent is water.

25 24. The method according to claim 1, wherein said plant pathogen is *Pseudomonas* species

 25. The method according to any of claims 1 to 11, wherein said extract of *Yucca* is provided in a formulation selected from the group consisting of a wettable powder, a dust, an
30 emulsifiable oil, and a granule.

26. The method according to claim 1, wherein said method comprises treating said soil with said composition.

5 27. The method according to claim 1, wherein said method comprises treating said plant with said composition.

28. The method according to any of claims 1 to 11, wherein said method further comprises contacting said pathogen with a bactericide, a fungicide, a chemical control agent, or a
10 biological control agent.

29. The method according to any of claims 1 to 11, wherein said method further comprises contacting said pathogen with a composition comprising acibenzolar-S-methyl.

15 30. A composition for controlling a plant pathogen, wherein said composition comprises a pesticidally effective amount of an extract from a Yucca plant.

31. The composition according to claim 30, wherein said composition comprises from about 0.01% to about 10% extract of Yucca.
20

32. The composition according to claim 30, wherein said composition comprises from about 0.1% to about 5% of extract of Yucca.

33. The composition according to claim 30, wherein said composition comprises about
25 1% extract of Yucca.

34. The composition according to any of claims 30 to 33, wherein said Yucca plant is *Yucca schidigera*.

35. The composition according to any of claims 30 to 33, wherein said composition comprises an alcohol.

36. The composition according to claim 35, wherein said alcohol is ethanol.

5

37. The composition according to claim 36, wherein said composition comprises from about 0.1% to about 5% of ethanol.

38. The composition according to any of claims 30 to 37, wherein said composition
10 comprises a detergent.

39. The composition according to claim 38, wherein said composition comprises from about 0.01% to about 5% of detergent.

15 40. The composition according to claim 30, wherein said composition comprises about 1% of extract of *Yucca*, about 1% ethanol, and about 0.05% detergent.

41. The composition according to any of claims 30 to 40, wherein said plant pathogen causes bacterial wilt disease in plants.

20

42. The composition according to any of claims 30 to 40, wherein said plant pathogen is *Ralstonia solanacearum*.

43. The composition according to claim 42, wherein said *Ralstonia solanacearum* is race
25 1, race 2, race 3, race 4, or race 5 of *Ralstonia*.

44. The composition according to claim 42, wherein said *Ralstonia solanacearum* is race 1, biovar 1.

45. The composition according to any of claims 30 to 40, wherein said composition further comprises a bactericide or a fungicide.

46. The composition according to any of claims 30 to 40, wherein said composition
5 further comprises a chemical control agent or a biological control agent.

47. The composition according to claim 46, wherein said chemical control agent is selected from the group consisting of benomyl, borax, captafol, captan, chlorothalonil, various formulations containing copper; various formulations containing zinc; dichlone, dicloran, iodine,
10 various ergosterol biosynthesis inhibiting fungicides including but not limited to fenarimol, imazalil, myclobutanil, propiconazole, prochloraz, terbutrazole, flusilazole, triadimefon, and tebuconazole; folpet, iprodione, mancozeb, maneb, metalaxyl, oxycarboxin, oxytetracycline, PCNB, pentachlorophenol, quinomethionate, sodium arsenite, sodium DNOC, sodium hypochlorite, sodium phenylphenate, streptomycin, sulfur, thiabendazole, thiophanate-methyl,
15 triforine, vinclozolin, zineb, ziram, tricyclazole, cymoxanil, blasticidin, and validimycin.

48. The composition according to claim 46, wherein said biological control agent is selected from the group consisting of *Bacillus* sp., *Trichoderma* sp., *Erwinia* sp., *Pichia* sp., *Candida* sp., *Cryptococcus* sp., *Talaromyces* sp., *P. fumosoreus*, *B. bassiana*, *Chaetomium* sp.,
20 *Gliocladium* sp., *Aureobasidium* sp., *Dabaryomyces* sp., *Exophilia* sp., *Ampelomyces* sp., and *Mariannaea* sp.

49. The composition according to any of claims 30 to 40, wherein said extract of *Yucca* is provided in a solvent.
25

50. The composition according to claim 49, wherein said solvent is water.

51. The composition according to claim 30, wherein said plant pathogen is *Pseudomonas* species.
30

52. The composition according to any of claims 30 to 40, wherein said extract of Yucca is provided in a formulation selected from the group consisting of a wettable powder, a dust, an emulsifiable oil, or a granule.

5 53. The composition according to any of claims 30 to 40, wherein said composition further comprises acibenzolar-S-methyl.

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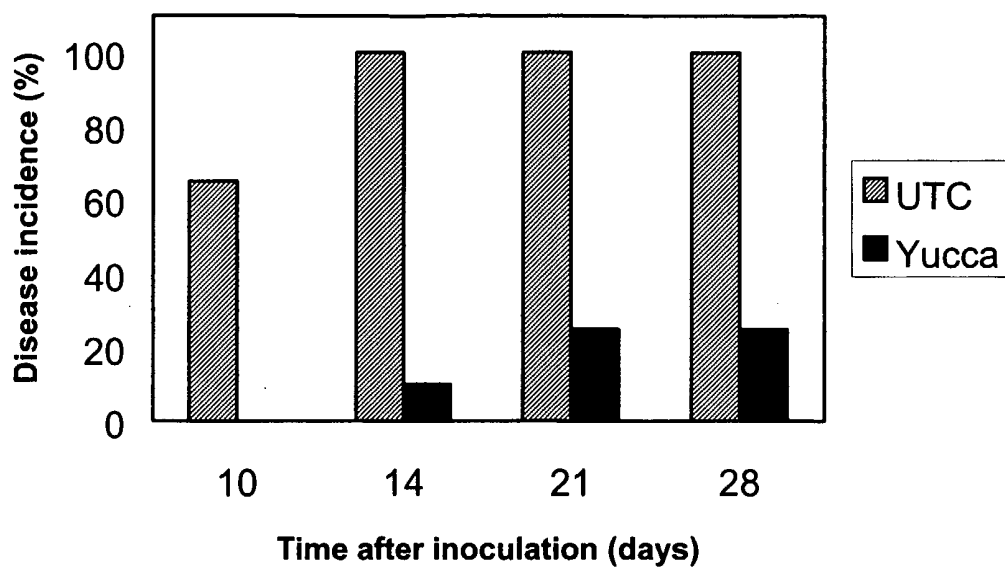


FIG. 1



FIG. 2

FIG. 3A

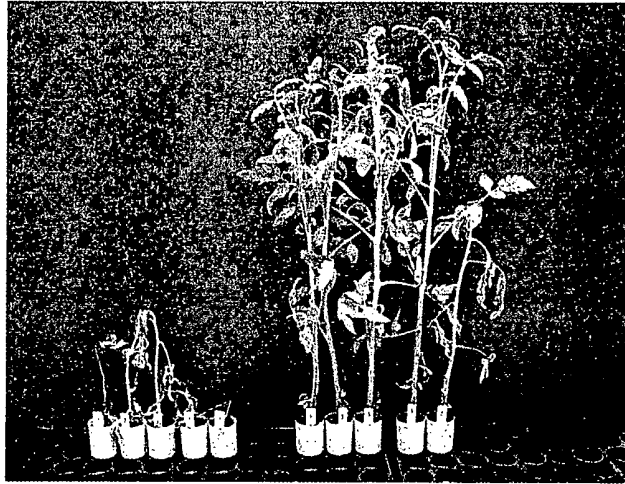


FIG. 3B



FIG. 3C

