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Canadian Intellectual Property Office

CA 2769506 C 2019/03/26

(11)(21) **2 769 506**

(12) **BREVET CANADIEN
CANADIAN PATENT**

(13) **C**

(86) Date de dépôt PCT/PCT Filing Date: 2010/07/29
(87) Date publication PCT/PCT Publication Date: 2011/02/03
(45) Date de délivrance/Issue Date: 2019/03/26
(85) Entrée phase nationale/National Entry: 2012/01/27
(86) N° demande PCT/PCT Application No.: US 2010/043612
(87) N° publication PCT/PCT Publication No.: 2011/014596
(30) Priorité/Priority: 2009/07/30 (US61/230,102)

(51) Cl.Int./Int.Cl. *A01N 35/06* (2006.01), *A01N 37/42* (2006.01), *A01N 37/46* (2006.01), *A01N 37/50* (2006.01), *A01N 43/16* (2006.01), *A01N 43/36* (2006.01), *A01N 43/42* (2006.01), *A01N 43/54* (2006.01), *A01N 43/56* (2006.01), *A01N 43/653* (2006.01), *A01N 65/30* (2009.01), *A01P 3/00* (2006.01)

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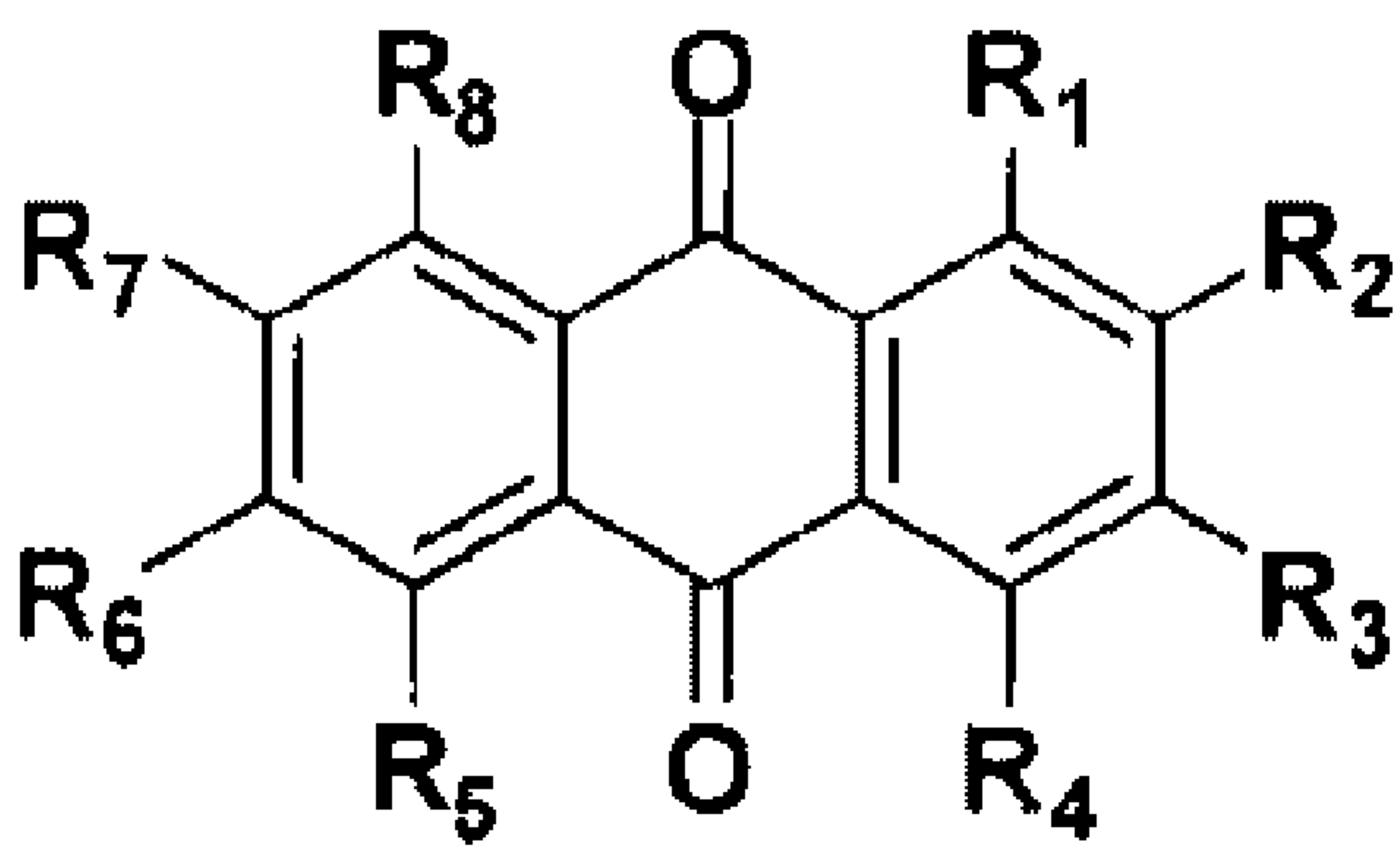
(54) Titre : MELANGE SYNERGETIQUE D'EXTRAIT DE PLANT DE REYNOUTRIA SACHALINENSIS RENFERMANT DU PHYSICON ET DE L'AZOXYSTROBIN CONTRE LE CHAMPIGNON CHEZ LES PLANTES

(54) Title: SYNERGISTIC MIXTURE OF A REYNOUTRIA SACHALINENSIS PLANT EXTRACT CONTAINING PHYSCION AND AZOXYSTROBIN AGAINST FUNGI IN PLANTS



(57) Abrégé/Abstract:
The disclosure provides a synergistic combination for use in modulation of phytopathogenic infection comprising
(a) an extract derived from a Reynoutria sachalinensis plant, wherein said extract comprises physcion and
(b) at least one chemical pesticide. In certain embodiments, the pesticide is selected from the group consisting of myclobutanil, quinoxyfen, azoxystrobin, triflumizole, acibenzolar-5-methyl, mefenoxam, propiconazole and fludioxonil. The amounts of said extract and at least one chemical pesticide in said combination provide for synergism of modulation of phytopathogenic infection, wherein the synergism is measured by determining E/Ee and E/Ee is at least 1.2.

Abstract

The disclosure provides a synergistic combination for use in modulation of phytopathogenic infection comprising

(a) an extract derived from a *Reynoutria sachalinensis* plant, wherein said extract comprises physcion and

(b) at least one chemical pesticide. In certain embodiments, the pesticide is selected from the group consisting of myclobutanil, quinoxyfen, azoxystrobin, triflumizole, acibenzolar-5-methyl, mefenoxam, propiconazole and fludioxonil. The amounts of said extract and at least one chemical pesticide in said combination provide for synergism of modulation of phytopathogenic infection, wherein the synergism is measured by determining E/Ee and E/Ee is at least 1.2.

SYNERGISTIC MIXTURE OF A *REYNOUTRIA SACHALINENSIS* PLANT EXTRACT CONTAINING PHYSCION AND AZOXYSTROBIN AGAINST FUNGI IN PLANTS

TECHNICAL FIELD

Disclosed herein are combinations, compositions and methods of use for modulating plant pathogen infection using plant extracts containing anthraquinone derivatives which induce resistance to plant phytopathogens and an antimicrobial agent, a biological control agent and/or a surfactant having fungicidal activity.

BACKGROUND

Plant resistance to plant pathogens

Plants have evolved highly effective mechanisms for resistance to disease caused by infectious agents, such as bacteria, fungi and viruses. This resistance can be caused by several mechanisms, the best known of which are the systemic acquired resistance (SAR; Ross, 1961; Durrant and Dong, 2004) and induced systemic resistance (ISR; van Loon et al., 1998). In the most simple case, the inducer is the plant pathogen itself, in other cases, the inducer can be either a chemical compound (salicylic acid, benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester also known as BTH) or physical impact such as water or heat stress (Walters et al., 2005). It appears that induced systemic resistance depends upon a gradual expression and persistence of a low level of metabolic perturbation. Unlike elicitors of phytoalexin accumulation, which elicit at the site of application and may be responsible for localized protection, inducers of systemic resistance sensitize the plant as a whole to respond rapidly after infections. These responses include phytoalexin accumulation, lignification and enhanced activities of chitinase and glucanase.

Extract from giant knotweed (*Reynoutria sachalinensis*) sold as MILSANA® and REGALIA® by Marrone Bio Innovations, Inc.) provides control of powdery mildew and other plant diseases on cucurbits and other crops mainly by inducing an accumulation of fungitoxic phenolic compounds in the plant (Daayf et al., 1995; Wurms et al. 1999; Schmitt, 2002). Recently, formulated giant knotweed extract has also shown great efficiency in inducing resistance in various crops and plant pathogens including wheat powdery mildew (Vechet et al., 2009). Besides the ISR mode of action, the formulated *R. sachalinensis* extract has recently also been shown to have a direct fungistatic effect against wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*; Randoux et al., 2008).

Fungicide resistance

Fungicide resistance is a common phenomenon in pests including plant pathogens. When a fungicide, especially those with single-site mode of action, is frequently used, the targeted pathogen can adapt to the fungicide due to high selection pressure. It is estimated that pests can develop resistance to pesticides within 5-50 generations (May, 1985). Most plant pathogens fit in this range in one growth season and thus can develop fungicide resistance quickly. For example, it only took one year for benomyl lost efficacy for control of cucurbit powdery mildew after its first registration for commercial use (McGrath, 2001).

Quinone outside inhibitors (also known as QoI fungicides or strobilurins) has been widely used to control agriculturally important fungal pathogens since their introduction in 1996. Strobilurins block the respiration pathway by inhibiting the cytochrome bc1 complex in mitochondria, thereby blocking the electron transfer process in the respiration chain and causing an energy deficiency due to lack of adenosine triphosphate (ATP) (Bartlett et al., 2002). Strobilurins and other fungicides with a single-site mode of action such as demethylation inhibitors (DMI) are prone to resistance development among plant pathogens. To date, several plant pathogenic fungi have developed field resistance to strobilurins (Tuttle McGrath, 2003; Fraaije et al., 2003) and DMI fungicides (Schnabel et al., 2004), and considerable effort has been made worldwide to develop appropriate resistance management strategies with detailed recommendations of how to combine fungicides and other antifungal compounds in programs and rotations to minimize the risk of resistance development (Tuttle McGrath, 2006; Wyenandt et al., 2009).

Methods to control fungicide resistance

The most common strategy to manage fungicide resistance is to use site-specific fungicides that are prone to resistance development in a combination (pre-mix or tank mix). Besides resistance management, tank mixes also offer a compensatory mechanism in case of a failure of one fungicide as well as a way to reduce the dose to reduce selection pressure on pathogens (van den Bosch and Gilligan, 2008). In some cases, the combination of single and multisite fungicides in a tank mix or in rotation can provide additive or even synergistic interactions (Gisi, 1996). Holb and Schnabel (2008) were able to show improved control of brown rot (*Monilinia fructicola*) in a field study with a tank mix of a DMI fungicide and elemental sulfur, and Reuveni (2001) demonstrated the benefits of using strobilurins and polyoxin B fungicides in combination with sulfur to control powdery mildew in nectarines.

Plant defense inducers such as the extract of *R. sachalinensis* have been tested in tank mixes and rotations with other SAR/ISR products as well as with biocontrol agents (BCA) (Hafez et al., 1999; Belanger and Benyagoub, 1997; Schmitt et al., 2002; Schmitt and Seddon, 2005; Bardin et al., 2008). The purpose of these studies has mainly been to demonstrate the compatibility of different types of plant extracts with biocontrol agents. Konstatinidou-Doltsinis et al. (2007) tested the *R. sachalinensis* product in a rotation with *Pseudozyma flocculosa* product against powdery mildew on grapes, and found that alternated application of both products improved the efficacy of *R. sachalinensis*. In the same study, alternation of sulfur and *R. sachalinensis* in a rotation did not have a beneficial effect. Belanger and Benyagoub (1997) found that a yeast-like fungus, *Pseudozyma flocculosa*, was compatible with *R. sachalinensis* when used against cucumber powdery mildew in a greenhouse. Similarly, Bokshi et al. (2008) evaluated the combined effect of an acquired systemic resistance activator benzothiadiazole and MILSANA® against cucumber powdery mildew, and found that MILSANA® used in a rotation with benzothiadiazole provided an effective control measure against powdery mildew in the field. However, based on the disease severity and yield data collected, it was not possible to determine whether the positive effect was additive or synergistic.

Pesticide synergism has been defined as "the simultaneous action of two or more compounds in which the total response of an organism to the pesticide combination is greater than the sum of the individual components" (Nash, 1981). Hence, when fungicides interact synergistically, a high level of disease control is achieved with less than label rates of each individual fungicide. Usually, the best effect is achieved with combinations of fungicides with different modes of action (MOA), but synergy has also been demonstrated in combined use of products with similar mode of action (De Waard, 1996). Fungicide synergism has been demonstrated mostly in laboratory studies (Samoucha and Cohen, 1984; Gisi, 1996) but in some cases (Karaogladinis and Karadimos, 2006; Burpee and Latin, 2008) synergism has also been found in the field studies. Additionally, synergism of antifungal compounds other than fungicides (bicarbonates and refined petroleum distillate) has been demonstrated against rose powdery mildew and black spot (Horst et al., 1992).

SUMMARY

Disclosed and claimed is a combination comprising: (a) an extract derived from a plant, wherein said extract contains one or more anthraquinone derivatives which induce plant resistance to phytopathogens (also referred to as "plant pathogens") and (b) one or more anti-phytopathogenic agents selected from the group consisting of: (i) a non-benzodiathiazole, non-Vitamin E, non-organophosphorusanti-microbial agent, which lacks or in other words does not contain non-elemental, non-wettable sulfur, (ii) a surfactant having fungicidal activity and (iii) a non-*Bacillus*, non-*Pseudomonas*, non-*Brevabacillus*, non-*Lecanicillium*, non-*Ampelomyces*, non-*Phoma*, non-*Pseudozyma* biological control agent (e.g., an agent derived from *Streptomyces* sp., *Burkholderia* sp., *Trichoderma* sp., *Gliocladium* sp. or a natural oil or oil-product having fungicidal and/or insecticidal activity).

In a specific embodiment, the combination comprises: (a) an extract derived from the family *Polygonaceae* and (b) a non-benzodiathiazole, non-Vitamin E, non-organophosphorus anti-fungal and/or antibacterial agent, which lacks or does not contain non-elemental or non-wettable sulfur.

In one specific embodiment, the combination comprises (a) an extract derived from the family *Polygonaceae* (e.g. *Reynoutria sachalinensis*) and (b) a single site fungicide and/or multi-site fungicide which may include but is not limited to myclobutanil, quinoxyfen, azoxystrobin, acibenzolar-*S*-methyl, mefenoxam, triflumizole, fludioxonil, propiconazole.

In another specific embodiment, the combination comprises (a) an extract derived from the family *Polygonaceae* (e.g. *Reynoutria sachalinensis*) and (b) a natural oil or oil-product having fungicidal and/or insecticidal activity.

In yet another particular embodiment, the combinations are compositions, particularly compositions for use in modulating phytopathogenic or fungal infection. The invention is further directed to the use of the extract and anti-phytopathogenic agents in formulating these compositions.

The invention is additionally directed to a synergistic combination for use in modulating phytopathogenic infection comprising (a) an extract derived from a plant, wherein said plant contains anthraquinone derivatives that induce plant resistance to phytopathogens and (b) a non-Vitamin E, non-organophosphorus antimicrobial agent (e.g., anti-fungal and/or antibacterial agent), which lacks or does not contain non-elemental or non-wettable sulfur. In a particular embodiment, the antimicrobial agent is a benzodiathiazole (e.g., acibenzolar-*S*-methyl), a triazole (e.g., propiconazole) or a strobilurin (e.g., azoxystrobin).

The above mentioned combinations may also be formulated into compositions.

The invention is further directed to a method for modulating phytopathogenic infection in a plant comprising applying to the plant and/or seeds thereof and/or substrate used for growing said plant an amount of the combinations of the present invention set forth above effective to modulate said phytopathogenic infection.

In a particular embodiment, the invention is directed to a method for modulating fungal and/or bacterial infection in a plant comprising applying to the plant and/or seeds thereof and/or substrate used for growing said plant an amount of the combinations of the present invention effective to modulate said fungal and/or bacterial infection.

The extract and said anti-phytopathogenic agents (e.g., anti-fungal and/or antibacterial agents) may be administered sequentially, concurrently or in combination intermittently. As defined herein "phytopathogenic infection" means infection of a plant by plant pathogenic bacteria, fungi, insects, nematodes and/or mollusks.

The invention is further directed to a method for decreasing the resistance of a phytopathogen (e.g, fungus and/or bacteria) to (i) a non-Vitamin E, non-organophosphorus anti-microbial agent, which lacked or in other words did not contain non-elemental or non-wettable sulfur, (ii) a surfactant having fungicidal activity and/or (iii) a non-*Bacillus*, non-*Pseudomonas*, non-*Brevabacillus*, non-*Lecanicillium,* non-*Ampelomyces,* non-*Phoma*, non-*Pseudozyma* biological control agent (e.g., an agent derived from *Streptomyces* sp., *Burkholderia* sp., *Trichoderma* sp., *Gliocladium* sp. or a natural oil or oil-based product having fungicidal and/or insecticidal activity) comprising applying to a plant in need thereof an amount of the combinations of the present invention effective to decrease the emergence of said resistance. In a particular embodiment, the invention is directed to a method for decreasing the resistance of a fungus and/or bacteria to a non-elemental or non-wettable, sulfur, non-benzodiathiazole, non-Vitamin E, non-organophosphorus anti-fungal and/or antibacterial agent. In yet another particular embodiment, the invention is directed to a method for decreasing resistance of a fungus and/or bacteria to a natural oil or oil-based product having fungicidal and/or insecticidal activity using the combination of the present invention. This may be accomplished by decreasing the frequency or rate of emergence. The resistance of a phytopathogen to the above-mentioned anti-pathogenic agents may be reduced by at least 50%.

The invention is further directed to the use of (a) an extract derived from a plant, wherein said extract contains anthraquinone derivatives which induce plant resistance to phytopathogens, and (b) one or more anti-phytopathogenic agents selected from the group consisting of: (i) a non-benzodiathiazole, non-Vitamin E, non-organophosphorus anti-microbial agent which lacks nonelemental, non-wettable sulfur, (ii) a surfactant having fungicidal activity and (iv) a non-*Bacillus*, non-*Pseudomonas*, non-*Brevabacillus*, non-*Lecanicillium,* non-*Ampelomyces*, non-*Phoma,* non-*Pseudozyma* biological control agent for the manufacture of a composition for modulating phytopathogenic infection as well as the use of (a) an extract derived from a plant, wherein said plant contains anthraquinone derivatives that induce plant resistance to phytopathogens and (b) a benzodiathiazole anti-microbial agent for the manufacture of a synergistic composition for modulating phytopathogenic infection.

The invention is further directed to a kit comprising (a) an extract derived from the family *Polygonaceae* and (b) one or more anti-phytopathogenic agents selected from the group consisting of: (i) a non-benzodiathiazole, non-Vitamin E, non-organophosphorus, anti-microbial agent, which lacks a non-elemental or non-wettable sulfur, (ii) a surfactant having fungicidal activity and (iii) a non-*Bacillus*, non-*Pseudomonas*, non-*Brevabacillus,* non-*Lecanicillium,* non-*Ampelomyces,*on-*Phoma,* non-*Pseudozyma* biological control agent. This kit may further comprise packaging instructions.

DETAILED DESCRIPTION OF SPECIFIC EMBODIMENTS

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. Further, although this invention has been described with reference to specific embodiments, the details thereof are not to be construed as limiting, as it is obvious that one can use various equivalents, changes and modifications and still are within the scope of the present invention.

Various references are cited throughout this specification.

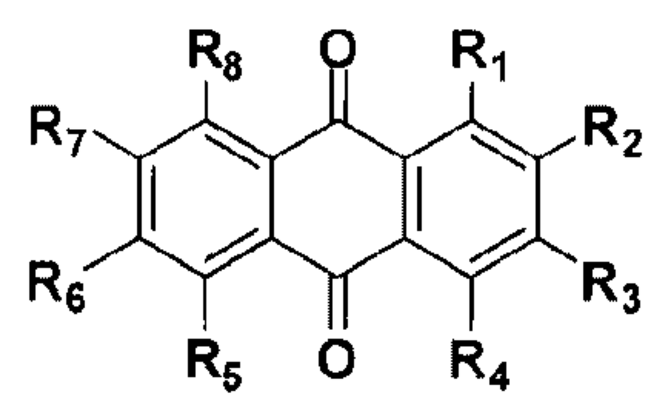
It must be noted that as used herein and in the appended claims, the singular forms "a," "and" and "the" include plural references unless the context clearly dictates otherwise. For example, “a fungus” also encompasses “fungi”.

As defined herein, the term “modulate” is used to mean to alter the amount of phytopathogenic infection or rate of spread of phytopathogenic infection.

Extracts

The plant extracts used in the combinations, compositions and methods of the present invention contain anthraquinone derivatives as biochemical agricultural products for use against plant pests, particularly plant phytopathogens such as plant pathogenic bacteria, fungi, insects, nematodes and/or as a molluscicide. "Contain" also encompasses extracts that produce said anthraquinone derivatives. In a particular embodiment, the anthraquinone derivative(s) of the present invention which is used in compositions and methods of the present invention is (are) the major active ingredients or one of the major active ingredients.

Anthraquinone derivatives include but are not limited to physcion, emodin, chrysophanol, ventiloquinone, emodin glycoside, chrysophanol glycoside, physcion glycoside, 3, 4-dihydroxy-1-methoxy anthraquinone-2-corboxaldehyde, damnacanthal. These derivatives share a similar structure as follows:



Where R1, R2, R3, R4, R5, R6, R7 and R8 are hydrogen, hydroxyl, hydroxylalkyl, halogen, carboxyl, alkyl, alkyoxyl, alkenyl, alkenyloxyl, alkynyl, alkynyloxyl, heterocyclyl, aromatic, or aryl group, sugars such as glucose.

In a particular embodiment, the invention is directed to anthraquinone derivatives that are contained in extracts derived from plant families including but not limited to Polygonaceae, Rhamnaceae, Fabaceae, Asphodelaceae, and Rubiaceae so on. These compounds can be from any part of plants such as leaf, stem, bark, root and fruits. Plant materials can be wet and dry, but preferably dry plant materials. To meet the biochemical agricultural products, solvents and processes that are used in the extraction and purification must meet the requirements of National Organic Program (NOP).

In a more particular embodiment, the plant extract is derived from a member of the *Polygonaceae* family. As defined herein, "derived from" means directly isolated or obtained from a particular source or alternatively having identifying characteristics of a substance or organism isolated or obtained from a particular source. In a particular embodiment, extract in said combination contains at anthraquinone derivatives physcion and optionally emodin. Members of the Polygonaceae family include but are not limited to *Acetosella, Antigonon, Aristocapsa, Bilderdykia, Brunnichia, Centrostegia, Chorizanthe, Coccoloba, Coccolobis, Coccolobo, Corculum, Dedeckera, Delopyrum, Dentoceras, Dodecahema, Emex, Eriogonum, Fafopyrum, Fagopyrum, Fallopia, Gilmania, Goodmania, Harfordia, Hollisteria, Koenigia, Lastarriaea, Mucronea, Muehlenbeckia, Nemacaulis, Oxyria, Oxytheca, Perscarioa,* *Persicaria, Pleuropterus, Podopterus, Polygonella, Polygonum, Pterostegia, Rheum, Rumex, Ruprechtia, Stenogonum, Systenotheca, Thysanella, Tovara, Tracaulon, Triplaris* and even more particular embodiment, the extract may be derived from a *Reynoutria* (alternately referred to as *Fallopia*) sp. or *Rheum* species. In a most particular embodiment, the extract is derived from *Reynoutria sachalinensis*.

Anti-Phytopathogenic agents

The formulated extract (such as products marketed under trade names REGALIA® and MILSANA®) can then be used in combination with other anti-phytopathogenic agents plant extracts, biopesticides, inorganic crop protectants (such as copper), surfactants (such as rhamnolipids; Gandhi et al., 2007) or natural oils such as paraffinic oil and tea tree oil possessing pesticidal properties or chemical fungicides or bactericides with either single site, multisite or unknown mode of action. As defined herein, an "anti-phytopathogenic agent" is an agent which modulates the growth of a plant pathogen on a plant or alternatively prevents infection of a plant by a plant pathogen. A plant pathogen includes but is not limited to a fungus, bacteria, virus, insects, nematodes and/or mollusca.

In a particular embodiment, the anti-phytopathogenic agent is a biopesticide alternatively referred to as a biocontrol agent. This biocontrol agent is in a more particular embodiment a non-*Bacillus*, non-*Pseudomonas*, non-*Brevabacillus*, non-*Lecanicillium*, non-*Ampelomyces*, non-*Phoma*, non-*Pseudozyma* biological control agent is an agent derived from *Streptomyces* sp., *Burkholderia* sp., *Trichoderma* sp., *Gliocladium* sp. Alternatively, the agent is a natural oil or oil-product having fungicidal and/or insecticidal activity (e.g., paraffinic oil, tea tree oil, lemongrass oil, clove oil, cinnamon oil, citrus oil, rosemary oil).

As noted above, the anti-phytopathogenic agent may be a single site anti-fungal agent which may include but is not limited to benzimidazole, a demethylation inhibitor (DMI) (e.g., imidazole, piperazine, pyrimidine, triazole), morpholine, hydroxypyrimidine, anilinopyrimidine, phosphorothiolate, quinone outside inhibitor, quinoline, dicarboximide, carboximide, phenylamide, anilinopyrimidine, phenylpyrrole, aromatic hydrocarbon, cinnamic acid, hydroxyanilide, antibiotic, polyoxin, acylamine, phthalimide, benzenoid (xylylalanine). In a more particular embodiment, the antifungal agent is a demethylation inhibitor selected from the group consisting of imidazole (e.g., triflumizole), piperazine, pyrimidine and triazole (e.g.,bitertanol, myclobutanil, penconazole, propiconazole, triadimefon, bromuconazole, cyproconazole, diniconazole, fenbuconazole, hexaconazole, tebuconazole, tetraconazole, propiconazole). In a most particular embodiment, the antifungal agent is myclobutanil. In yet another particular embodiment, the antifungal agent is a quinone outside inhibitor (e.g., strobulurin). The strobulurin may include but is not limited to azoxystrobin, kresoxim-methyl or trifloxystrobin. In yet another particular embodiment, the anti-fungal agent is a quinone, e.g., quinoxyfen (5,7-dichloro-4-quinolyl 4-fluorophenyl ether).

In yet a further embodiment, the antimicrobial agent is a multi-site non-inorganic, chemical fungicide selected from the group consisting of a nitrile (e.g., chloronitrile or fludioxonil), quinoxaline, sulphamide, phosphonate, phosphite, dithiocarbamate, chloralkythios, phenylpyridin-amine, cyano-acetamide oxime.

In yet another embodiment, the anti-phytopathogenic agent is an anti-bacterial agent. This anti-bacterial agent includes but is not limited to carbamates, organophosphates, cyclodiene organochlorides, phenylpyrazoles, pyrethroids, pyrethrins, neonicotinoids, nitroguanadines, nicotine, Spinosyn, glycosides, juvenile hormone analogues and other insect growth regulators, pyridine azomethine, pyridine carboxamide, tetrazine, thiazolidinone, 2,4-diphenyloxzoline derivatives, organotin, pyrrole, buprofezin, hydramethylnon, naphtoquinon derivatives, pyridazinone, phenoxypyrazole, tetronic acid, carbazate, rotenone, organochlorine-diphenylaliphatics.

Uses

The said plant extract or formulated product can be used simultaneously with the other component or components in a tank mix or in a program (sequential application called rotation) with predetermined order and application interval during the growing season. When used in a combination with the above-mentioned pesticidal products, at concentration lower than recommended in the product label, the combined efficacy of the two or more products (one of which is the said plant extract) is in a preferred embodiment, higher than each individual component's effect added together. Hence, the pesticidal effect is enhanced by synergism between these two (or more) products, and the risk for the development of pesticide resistance among the plant pathogenic strains is reduced.

Target plants to be protected within the scope of the present invention comprise, for example, the following species of plants: cereals (wheat, barley, rye, oats, rice, sorghum and related crops), beet (sugar beet and fodder beet), pommes and soft fruit (apples, pears, plum, peaches, almonds, cherries, strawberries, raspberries and blackberries), leguminous plants (beans, lentils, peas and soybeans), oil plants (rape, mustard, poppy, olives, sunflowers, coconuts, castor oil plants, cocoa beans and ground nuts), cucurbits (cucumber, melons, pumpkins, eggplant), fiber plants (cotton, flax, hemp and jute), citrus fruit (oranges, lemon, grapefruits and mandarins), vegetables (spinach, lettuce, asparagus, cabbages, carrots, onions, tomatoes, potatoes and paprika), lauraceae (avocadoes, cinnamon and camphor) or plants such as maze, tobacco, nuts, coffee, sugar cane, tea, vines, hops, bananas and natural rubber plants, as well as ornamentals (composites), areas of grass or general low cover crops which counteract erosion or desiccation of the soil and are useful in cultures of trees and perennials (fruit plantations, hop plantations, maze fields, vineyards, etc.).

The preferred method of applying combinations of products in the present invention is a foliar application (spraying, atomizing, dusting, scattering or pouring) with or without a carrier. The number of applications and the rate of application depend on the risk of infestation by a pathogen. For example, the foliar pesticidal treatments with combinations and mixtures covered in this patent can be made once in every 7 to 14 days at 25 to 10,000-fold lower rates than recommended in the product label. Product mixtures and combinations targeted in the present invention may also be applied to seeds by impregnating the seeds either with a liquid formulation containing the active ingredient or coating them with a solid formulation. In special cases, further types of application are also possible. These include soil drench or selective treatment of plant stems or buds.

The mixtures of the present invention and, where appropriate, a solid or liquid adjuvant are prepared in known manner. For example, the mixtures may be prepared by homogeneously mixing and/or grinding the active ingredients with extenders such as solvents, solid carriers and, where appropriate, surface-active compounds (surfactants).The compositions may also contain further ingredients such as stabilizers, viscosity regulators, binders, adjuvants as well as fertilizers or other active ingredients in order to obtain special effects.

EXAMPLES

As will be set forth below, in satisfaction of the foregoing objects and advantages, examples of methods for increasing the efficacy of two or more products by using them at rates that produce synergistic or additive effects. The compositions and methods described here have been proven effective in reducing the disease incidence and severity in greenhouse-grown cucumbers (*Cucumis sativus*) but the concept can be used effectively for other plant varieties and species. The compositions and methods are particularly effective against cucumber powdery mildew but they can be applied to other fungal, bacterial, and viral plant diseases as well such as grey mold, leaf spots, bacterial wilt, scab, anthracnose, tobacco mosaic virus etc.

Materials and Methods

Powdery Mildew (Examples I-V and X-XI)

The studies were conducted in a green house. Experimental design of the studies for synergism followed the Burpee and Latin (2008). Powdery mildew, caused by *Sphaerotheca fuliginea*, was used for investigating the efficacy of the treatments.

Cucumber seeds cv. “SMR 58” (Irwin & Sons Ag Supply, Inc. Cheshire, OR) were grown in plastic 4-inch pots with potting soil mix (Rod McLellan Company, Marysville, OH). The plants were treated at 2-true leaf stage. The compounds at various rates were sprayed with a 2-oz mist sprayer at 2 ml per plant at upper side and 1 ml at lower side of the leaves. The treated plants were left for 3-4 hours under florescent light to dry before inoculation.

A conidia suspension of *Sphaerotheca fuliginea*, the causal agent of powdery mildew disease on cucumber, was prepared by cutting diseases leaves of the cucumber plants that are served for conservation of inoculum. The suspension were adjusted to 2.0×10^5 conidia per ml and applied with a 2-oz mist sprayer at 2 ml per plant on the upper side of the leaves. The inoculated plants were placed in a greenhouse and the treatments were arranged in randomized complete blocks with 4 to 6 replicates at 25°C to 30°C.

The disease severity (percentage area covered with colonies) of the first leaves were rated according to James (1971). Disease severity and percentage control were analyzed with analysis of variance (ANOVA) and means of the treatments were compared with Fisher’s Protected Least Difference (LSD) at p=0.05 level. Synergetic effect was calculated and analyzed with Limpel’s formula (Limpel et al., 1962; Richer, 1987).

Lettuce downy mildew (*Examples VI-VII*)

Synergy between MBI-106 and acibenzolar-*S*-methyl or mefenoxam which control a specific class of pathogens **Oomycetes** was tested with lettuce downy mildew following Su et al. (2004).

To prepare inoculum of downy mildew, lettuce seeds were placed on 5-mm petri-dishes with about 20-30 seeds in each and watered with sterile water, then supplied with ½ strength of Hoagland’s solution after germination. The petri dishes were placed at 20°C growth chamber for 7-10 days. The spores of *Bremia lactucae* was inoculated on lettuce cotyledons and cultured for 7-10 days at 15°C for sporulation to occur. Cotyledons with sporulation were cut off and placed in a falcon tube with sterile water. The cotyledons were vortexed for 15 seconds three times and filtered through a 100-uμm mesh to collect sporangia. The solution was adjusted to 0.5 to 1.0×10^5 spores/ml for inoculation.

Four test plants were seeded in each 2-in pots and then placed at 20°C for 7-10 days to grow cotyledons. Plants were ready for test when the first true leaf was emerging.

The lettuce plants were treated with the materials and left to dry or overnight. Then the plants were inoculated with sporangial solution. The treatments were arranged in a randomized complete block design with four replicates. The inoculated plants were placed in darkness in growth chamber for 48 hours and then incubated under 12-h light period at 15°C. Eight to ten days after inoculation, the cotyledons were rated for disease severity (percentage area covered with sporangiophores).

Seed treatment (*Examples VIII-IX*)

The experiments described below were conducted with soybean but similar procedures are used with other crops such as cereals, corn, cotton, and potatoes.

The experiments were conducted in a greenhouse following the procedure developed by Hwang et al. (2006). The isolate of *Rhizoctonia solani* was grown on potato dextrose agar plates and cultured for 5 days for inoculating oat or wheat grain. One liter of grain was soaked overnight at room temperature (25°C) and rinsed three times with tape water. The 8×12-in autoclave bags were filled with the grain up to 1/3 full were autoclaved at 121°C for 15 min. The autoclaved grain then was then inoculated with 5 pieces of 1×1 cm plug of the 5-day culture of *R. solani* per bag and cultured for 5 days at room temperature (25°C). The bags were left open in laminar flow hood to let the grain completely dry.

The blank seed sample of 10 g to be coated was placed in a flask. Coating agent SEPIRET® 1171-O (Becker Underwood Ltd., Ames, IA) was put into the flasks together with the compound of interest, and the flask was shaken vigorously so that the seed picked up the compound. The procedure was repeated with fresh seed sample of cvs. "White Lion" (Kitazawa Seed Co., Oakland, CA) or "Viking 2265" (Johnny's Selected Seeds, Winslow, ME) in the same flask for the treatments. After the seeds were coated, they were left to air dry before planting.

To prepare the soil, five hundred milliliters of the dried grains with inoculum were blended for 15 seconds three times and the powder was mixed with sterile sand at 1:1 (v/v) to dilute the inoculum. The sand mix was further used to prepare soil mix at various proportions 1:350 to 1:800 (inoculum: soil) for soybean to generate various disease levels in repeated tests.

The coated seeds were planted in the pots with infested soil. There were three replicates of each treatment which were arranged in a complete randomized block design and placed at 25 to 30°C in a greenhouse.

After 10 to 20 days, depending on disease pressure and temperature, emergence of each treatment was rated and compared. Biomass was measured by weighing the above-ground portion which was measured for each replicate of the plant material.

Example I. Synergistic effect between formulated *R. sachalinensis* extract and myclobutanil (test I).

MBI-106 (formulated *R. sachalinensis* extract marketed as REGALIA® SC by Marrone Bio Innovations, Inc., Davis, CA) diluted at 10-fold lower than label rates of 1500× and 2000× and myclobutanil (formulated as RALLY® 40W, Dow AgroSciences LLC, Indianapolis, IN) at concentrations of 0.25 ug/ml, 0.1 ug/ml, and 0.05 ug/ml (450 to 2,250-fold lower than recommended label rates) were applied alone or in tank mix.

Disease severity was significantly reduced in MBI-106 at 1500× and 2000× in combination with myclobutanil at 0.25 ug/ml than that when they were used alone (Table 1). Significant increase in control efficacy was found with the combination of MBI-106 2000× tank mixed with myclobutanil at rates of 0.25 ug/ml and 0.05 ug/ml (Table 2).

Example II. Synergistic effect between *R. sachalinensis* extract and myclobutanil (test II).

In a second test using MBI-106 (formulated as REGALIA® SC) and myclobutanil (formulated as RALLY® 40W), higher efficacy was found when MBI-106 at 2000× (10-fold lower than label rate) dilution in combination with myclobutanil at rates of 0.25, 0.1, and 0.05 ug/ml, and when MBI-106 at 1500× was tanked mixed with myclobutanil at 0.05 ug/ml (Table 3). Significant synergistic effect was displayed when MBI-106 diluted at 1500× and 2000× was combined with myclobutanil at the lowest rate of 0.05 ug/ml (Table 4).

Example III. Synergistic effect between formulated *R. sachalinensis* extract and quinoxyfen

MBI-106 diluted at 1500× or 2000× was applied alone or in combination with quinoxyfen (formulated as QUINTEC®, Dow AgroSciences LLC, Indianapolis, IN) at 0.05 and 0.01 ug/ml (2,000 to 10,000-fold lower than label rates) either alone or in a tank mix. The results are shown in Tables 5 and 6. The most significant synergistic effect and enhanced disease control was found at *R. sachalinensis* product dilution of 2000× with quinoxyfen at 0.01 ug/mL. Quinoxyfen has a new multi-site mode of action affecting G-proteins in early cell signaling.

Example IV. Synergistic effect between formulated *R. sachalinensis* extract and azoxystrobin

MBI-106 diluted at 1500× or 2000× was applied alone or in combination with azoxystrobin (formulated as QUADRIS®, Syngenta Corporation, Wilmington, DE) at a rate

0.25, 0.5, 1.0, 5.0, and 10 ug/mL (25 to 1020-fold lower than recommended label rates) either alone or in a tank mix. The results are shown in Tables 7 and 8. Of all tested combinations, mixes with *R. sachalinensis* extract at 2000× dilution and azoxystrobin at either 5.0 or 0.5 ug/mL provided the greatest synergy, and the fungicidal efficacy was significantly greater than expected compared to the single-compound use data.

Example V. Synergistic effect between formulated *R. sachalinensis* extract and triflumizole.

MBI-106 (formulated *R. sachalinensis* extract marketed as REGALIA® SC by Marrone Bio Innovations, Inc., Davis, CA) at 2500× dilution (10-fold lower than label rate) and triflumizole (formulated as PROCURE® 480SC, Chemtura Corporation, Middlebury, CT) at concentrations of 1.0 ug/ml, 0.5 ug/ml, and 0.25 ug/ml (150 to 600-fold lower than recommended label rates) were applied alone or in tank mix to.

Disease severity was significantly reduced ($P<0.0001$) in treatments sprayed with MBI-106 at 2500× dilution in combination with triflumizole compared with a treatment where MBI-106 was used alone (Table 9). Synergistic effect in efficacy was found at the combination of MBI-106 2500× tank mixed with triflumizole at rates of 1.0 ug/ml, 0.5 ug/ml, and 0.25 ug/ml (Table 10).

Example VI. Synergistic effect between *R. sachalinensis* extract and acibenzolar-*S*-methyl in controlling lettuce downy mildew.

MBI-106 (formulated *R. sachalinensis* extract marketed as REGALIA® SC by Marrone Bio Innovations, Inc., Davis, CA) was used alone at 200× dilution or in combination with acibenzolar-*S*-methyl (formulated as ACTIGARD®, Syngenta Crop Protection, Inc., Greensboro, NC) at concentrations of 25 ug/ml to control lettuce downy mildew.

Disease severity was significantly reduced ($p=0.0004$) in MBI-106 at 200× dilution in combination with acibenzolar-*S*-methyl compared to treatments in which they were used alone (Table 11). There is a synergistic effect in efficacy in the tank mix of MBI-106 200× and acibenzolar-*S*-methyl (Table 12).

Example VII. Synergistic effect between *R. sachalinensis* extract and mefenoxam in controlling lettuce downy mildew.

To investigate the synergistic effect of Reynoutria and MBI-106, formulated product REGALIA® ME by Marrone Bio Innovations, Inc., Davis, CA was used at 400× dilution rate in combination with mefenoxam (formulated as RIDOMIL GOLD®, Syngenta Crop Protection, Inc., Greensboro, NC) at concentrations of 37.5μg/ml, 75.0μg/ml, and 150μg/ml to control lettuce downy mildew.

Disease severity was significantly reduced ($p<0.0001$) in MBI-106 at 400× dilution in combination with mefenoxam compared to treatments in which they were used alone at each concentration (Table 13). Synergistic effect in efficacy of the tank mix of MBI-106 200× and mefenoxam was found in combinations of MBI-106 and each concentration of mefenoxam (Table 14).

Example VIII. *R. sachalinensis* extract as a seed treatment agent and its synergy with azoxystrobin in controlling *Rhizoctonia solani*.

Reynoutria sachalinensis was extracted with ethanol at 5% (w/w) and used for seed coating at 0.2117g/kg seed, either alone or in combination with azoxystrobin (QUADRIS®, Syngenta Crop Protection, Inc., Greensboro, NC) at 0.0298g/kg seed to control *Rhizoctonia solani* on soybean.

The emergence rate was higher in the inoculated seeds treated with MBI-106 compared to the inoculated untreated control, and when used in combination with azoxystrobin, the emergence rate was higher than when either product was used alone (Table 15). A synergistic effect was found when both materials were used in combination (Table 16).

Example IX. *R. sachalinensis* extract as a seed treatment agent and synergy with fludioxonil in controlling *Rhizoctonia solani*.

The ethanol extract of *R. sachalinensis* was also used at the rates of 0.03175g/kg seed and 0.635g/kg seed for seed coating, either alone or in combination with fludioxonil (formulated as Scholar® by Syngenta Crop Protection, Inc., Greensboro, NC) to control *Rhizoctonia solani* on soybean. The emergence rates and biomass were higher in the MBI-106 treated seeds compared to the inoculated untreated control and also higher when used in combination with fludioxonil at the rate of 0.0596g/kg seed (Table 17). Synergistic effect was found in the two rates of MBI-106 when used in combination with fludioxonil (Table 18).

Example X. Synergistic effect between formulated *R. sachalinensis* extract and propiconazole in controlling cucumber powdery mildew.

MBI-106 (formulated *R. sachalinensis* extract marketed as REGALIA® ME by Marrone Bio Innovations, Inc., Davis, CA) at 2500× dilution and propiconazole (formulated as PROPIMAX® EC, Dow AgroSciences LLC, Indianapolis, IN) at concentrations of 1.0 ug/ml were applied alone or in tank mix.

Disease severity was significantly reduced (P<0.0001) in treatments sprayed with MBI-106 at 2500× dilution in combination with propiconazole (Table 19). There is a synergistic effect in control efficacy in the combination of MBI-106 2500× tank mixed with propiconazole (Table 20).

Example XI. Synergistic effect between *R. sachalinensis* extract and quinoxifen (Test II) in controlling cucumber powdery mildew.

MBI-106 (formulated *R. sachalinensis* extract marketed as REGALIA® SC by Marrone Bio Innovations, Inc., Davis, CA) was used alone at 2000× dilution or in combination with azoxystrobin at three concentration 0.5, 0.25, and 0.1ug/ml to control cucumber powdery mildew.

The control efficacy in MBI-106 at 2000× dilution in combination with azoxystrobin treatments are higher that they are used alone (Table 21). Synergistic effect in control efficacy of the tank mix of MBI-106 2000× exists in combinations of MBI-106 and each concentration of azoxystrobin (Table 22).

Tables

Table 1. Disease severity and percentage control of MBI-106 and myclobutanil (RALLY® 40W) when used alone or in tank mix.[1]

Treatment	Dilution/Rate	Severity (%)[z]	Control (%)
Untreated control	N/A	98.3 a	0.0 e
myclobutanil	0.25 ug/ml	16.7 cd	83.1 bc
myclobutanil	0.1 ug/ml	95.0 a	3.2 e
myclobutanil	0.05 ug/ml	95.8 a	2.5 e
Reynoutria extract	1500×	15.8 cd	84.0 bc
Reynoutria extract	2000×	35.8 b	63.5 d
Reynoutria extract + myclobutanil	1500× 0.25 ug/ml	1.2 e	98.8 a
Reynoutria extract + myclobutanil	2000× 0.25 ug/ml	0.2 e	99.8 a
Reynoutria extract + myclobutanil	1500× 0.1 ug/ml	11.8 de	87.9 ab
Reynoutria extract + myclobutanil	2000× 0.1 ug/ml	27.5 bc	72.2 dc
Reynoutria extract + myclobutanil	1500× 0.05 ug/ml	17.5 cd	82.4 bc
Reynoutria extract + myclobutanil	2000× 0.05 ug/ml	20.8 cd	79.0 bc
		P<0.0001	P<0.0001

[1] Data in Column 3 (Severity (%)) are means of six replicates. Means with the same letter in a column are not significantly different according to Fisher's Protected Least Significant Difference (LSD) at P=0.05 level.

Table 2. Expected efficacy (Ee)[2] of each product combination, and the statistical significance of detected synergism between MBI-106 (REGALIA®SC) and myclobutanil (RALLY® 40W)

Treatment	**Dilution/Rate**	**Control (%)**	**E_e[3]**	**T-test**
Reynoutria extract + myclobutanil	1500× 0.25 ug/ml	98.8	97.3	n.s.
Reynoutria extract + myclobutanil	2000× 0.25 ug/ml	99.8	93.8	***
Reynoutria extract + myclobutanil	1500× 0.1 ug/ml	87.9	84.5	n.s.
Reynoutria extract +myclobutanil	2000× 0.1 ug/ml	72.2	64.7	n.s.[4]
Reynoutria extract + myclobutanil	1500× 0.05 ug/ml	82.4	84.4	n.s.
Reynoutria extract + myclobutanil	2000× 0.05 ug/ml	79.0	64.4	*[5]

[2] E_e is the Expected efficacy and is determined with the Limpel's formula $E_e=X + Y - (XY)/100$ (Limpel et al., 1962; Richer, 1987).

[3] Data are means of six replicates. Means with the same letter in a column are not significantly different according to Fisher's Protected Least Significant Difference (LSD) at P=0.05 level.

[4] n.s.: Not significant

[5] * and ***: Significant at $P<0.05$ and 0.001 respectively.

Table 3. Disease severity and percentage control of MBI-106 (REGALIA® SC) and myclobutanil (RALLY® 40W) when used alone or in tank mix in a repeated test.

Treatment	**Dilution/Rate**	**Severity (%)**[z6]	**Control (%)**
Untreated control	N/A	91.3 a	0.0 e
myclobutanil	0.25 ug/ml	53.8 bcd	41.3 dc
myclobutanil	0.1 ug/ml	80.0 ab	12.2 de
myclobutanil	0.05 ug/ml	93.8 a	-2.8 e
Reynoutria extract	1500×	20.0 ef	77.9 ab
Reynoutria extract	2000×	63.8 abc	29.8 cde
Reynoutria extract + myclobutanil	1500× 0.25 ug/ml	46.3 cde	48.8 bc
Reynoutria extract + myclobutanil	2000× 0.25 ug/ml	33.8 cdef	62.7 bc
Reynoutria extract + myclobutanil	1500× 0.1 ug/ml	33.8 cdef	62.6 bc
Reynoutria extract + myclobutanil	2000× 0.1 ug/ml	38.8 cde	57.3 bc
Reynoutria extract + myclobutanil	1500× 0.05 ug/ml	1.8 f	98.1 a
Reynoutria extract + myclobutanil	2000× 0.05 ug/ml	21.3 def	77.0 ab
		$P<0.0001$	$P<0.0001$

[6] Data are means of four replicates. Means with the same letter in a column are not significantly different according to Fisher's Protected Least Significant Difference (LSD) at P=0.05 level.

Table 4. Synergistic effect between MBI-106 (REGALIA® SC) and myclobutanil (RALLY® 40W) in a repeated test.

Treatment	Dilution/Rate	Control (%)	E_e [z]	T test
Reynoutria extract + myclobutanil	1500× 0.25 ug/ml	48.8	87.0	n.s.
Reynoutria extract + myclobutanil	2000× 0.25 ug/ml	62.7	58.8	n.s.
Reynoutria extract + myclobutanil	1500× 0.1 ug/ml	62.6	80.6	n.s.
Reynoutria extract + myclobutanil	2000× 0.1 ug/ml	57.3	38.3	n.s.
Reynoutria extract + myclobutanil	1500× 0.05 ug/ml	98.1	77.3	***
Reynoutria extract + myclobutanil	2000× 0.05 ug/ml	77.0	27.8	**

[z]E_e is the Expected efficacy and is determined with the Limpel's formula $E_e = X + Y - (XY)/100$ (Limpel et al., 1962; Richer, 1987).

** and ***: Significant at P<0.01 and 0.001 respectively.

n.s.: Not significant

Table 5. Disease severity and percentage control of MBI-106 (formulated *R. sachalinensis* extract) and quinoxyfen (QUINTEC®) when used alone or in tank mix.

Treatment	Dilution/Rate	Severity (%)	Control (%)
Untreated control	N/A	91.3 a	0
Reynoutria extract	1500×	20.0 c	77.93
Reynoutria extract	2000×	63.8 b	29.75
quinoxyfen	0.05 ug/ml	30.0 c	66.95
quinoxyfen	0.01 ug/ml	90.0 a	1.33
Reynoutria extract + quinoxyfen	1500× 0.05 ug/ml	25.0 c	72.65
Reynoutria extract + quinoxyfen	2000× 0.05 ug/ml	12.5 c	86.25
Reynoutria extract + quinoxyfen	1500× 0.01 ug/ml	22.5 c	75.28
Reynoutria extract + quinoxyfen	2000× 0.0 1 ug/ml	13.8 c	85.1
n=4, LSD		P<0.0001	P<0.0001

Data are means of four replicates. Means with the same letter in a column are not significantly different according to Fisher's Protected Least Significant Difference (LSD) at P=0.05 level.

Table 6. Synergistic effect between MBI-106 (Reynoutria extract, REGALIA® SC) and quinoxyfen (QUINTEC®)

Treatment	Dilution/Rate	Control (%)	E_e	T-test
Reynoutria extract + quinoxyfen	1500× 0.05 ug/ml	72.65	92.7	*
Reynoutria extract + quinoxyfen	2000× 0.05 ug/ml	86.25	76.8	n.s.
Reynoutria extract+ quinoxyfen	1500× 0.01 ug/ml	75.28	78.2	n.s.
Reynoutria extract + quinoxyfen	2000× 0.0 1 ug/ml	85.1	30.7	**

[Z] E_e is the Expected efficacy and is determined with the Limpel's formula $E_e=X + Y - (XY)/100$ (Limpel et al., 1962; Richer, 1987).

* and **: Significant at P<0.05 and 0.01 respectively.

n.s.: Not significant.

Table 7. Disease severity and percentage control of powdery mildew using MBI-106 (R. sachalinensis extract, REGALIA® SC) and azoxystrobin (QUADRIS®).

Treatment	Dilution/Rate	Severity (%)		Control (%)	
Untreated control	N/A	92.5	a	0.0	h
azoxystrobin	10.0 ug/ml	11.3	efg	87.6	abc
azoxystrobin	5.0 ug/ml	5.3	fg	94.3	ab
azoxystrobin	1.0 ug/ml	30.0	cdef	68.1	bcdef
azoxystrobin	0.5 ug/ml	48.8	bc	48.0	fg
azoxystrobin	0.25 ug/ml	45.0	bcd	51.8	efg
Reynoutria extract	1500×	11.3	efg	88.0	abc
Reynoutria extract	2000×	61.3	b	34.3	g
Reynoutria extract	2500×	48.8	bc	47.0	fg
Reynoutria extract + azoxystrobin	1500× 10.0 ug/ml	3.0	fg	96.8	ab
Reynoutria extract + azoxystrobin	2000× 10.0 ug/ml	4.0	fg	95.6	ab
Reynoutria extract + azoxystrobin	2500× 10.0 ug/ml	1.8	fg	98.1	ab
Reynoutria extract + azoxystrobin	1500× 5.0 ug/ml	1.0	g	98.9	ab
Reynoutria extract + azoxystrobin	2000× 5.0 ug/ml	0.8	g	99.2	a
Reynoutria extract + azoxystrobin	2500× 5.0 ug/ml	2.0	fg	97.9	ab
Reynoutria extract + azoxystrobin	1500× 1.0 ug/ml	11.3	efg	87.6	abc
Reynoutria extract + azoxystrobin	2000× 1.0 ug/ml	12.5	efg	86.3	abcd
Reynoutria extract + azoxystrobin	2500× 1.0 ug/ml	25.0	cdefg	73.4	abcdef
Reynoutria extract + azoxystrobin	1500× 0.5 ug/ml	16.3	defg	82.2	abcde
Reynoutria extract + azoxystrobin	2000× 0.5 ug/ml	11.3	efg	87.7	abc
Reynoutria extract + azoxystrobin	2500× 0.5 ug/ml	43.8	bcd	52.1	efg
Reynoutria extract +azoxystrobin	1500× 0.25 ug/ml	40.0	bcde	56.4	defg
Reynoutria extract + azoxystrobin	2000× 0.25 ug/ml	25.0	cdefg	73.0	abcdef
Reynoutria extract + azoxystrobin	2500× 0.25 ug/ml	36.3	bcde	60.5	cdefg
N=4		P<0.0001		P<0.0001	

Table 8. Synergistic effect between MBI-106 and azoxystrobin. Only treatments with statistically significant synergistic effect are included.

Treatment	Rate/Dilution	Control (%)	E_c	T-test
Reynoutria extract + azoxystrobin	2000× 10.0 ug/ml	95.6	91.9	*
Reynoutria extract + azoxystrobin	2500× 10.0 ug/ml	98.1	93.4	*
Reynoutria extract + azoxystrobin	2000× 5.0 ug/ml	99.2	96.3	**
Reynoutria extract + azoxystrobin	2000× 0.5 ug/ml	87.7	65.8	**

Table 9. Disease severity and percentage control of *R. sachalinensis* and triflumizole (PROCURE® 480SC) when used alone or in tank mix.

Treatment	Dilution/Rate	Severity (%)		Control (%)
Untreated control	N/A	97.5	a	0.0
Reynoutria extract	2500×	23.8	b	75.6
triflumizole	1.0 ug/ml	77.5	a	20.5
triflumizole	0.5 ug/ml	86.3	a	11.5
triflumizole	0.25 ug/ml	95.0	a	2.6
Reynoutria extract + triflumizole	2500× 1.0 ug/ml	6.3	b	93.6
Reynoutria extract + triflumizole	2500× 0.5 ug/ml	15.0	b	84.6
Reynoutria extract + triflumizole	2500× 0.25 ug/ml	11.5	b	88.2

Data in Column 3 (Severity (%)) are means of four replicates. Means with the same letter in a column are not significantly different according to Fisher's Protected Least Significant Difference (LSD) at P=0.05 level.

Table 10. The expected efficacy (E_e) of each product combination, control efficacy (E), and the synergistic effect ($E/E_e > 1.0$) between MBI-106 (REGALIA® SC) and triflumizole (PROCURE® 480SC).

Tank mix	Dilution/Rate	Control (%)	E_e	E/E_e
Reynoutria extract + triflumizole	2500× 1.0 ug/ml	93.6	80.6	1.2
Reynoutria extract + triflumizole	2500× 0.5 ug/ml	84.6	78.4	1.1
Reynoutria extract + triflumizole	2500× 0.25ug/ml	88.2	76.2	1.2

E_e is the Expected efficacy and is determined with the Limpel's formula $E_e = X + Y - (XY)/100$ (Limpel et al., 1962; Richer, 1987).

Table 11. Disease severity and percentage control of MBI-106 (REGALIA® SC) and

Treatment	Dilution/Rate	Severity (%)	Control (%)
Untreated control	N/A	90.0 a	0.0
acibenzolar-*S*-methyl	25 ug/ml	68.9 ab	23.4
Reynoutria extract	200×	57.0 b	36.6
Reynoutria extract + acibenzolar-*S*-methyl	200× 25 ug/ml	14.2 c	84.2

acibenzolar-S-methyl (ACTIGARD®) when used alone or in tank mix.

Table 12. The expected efficacy (E_e) of each product combination, control efficacy (E), and the synergistic effect ($E/E_e > 1.0$) between MBI-106 (REGALIA® SC) and acibenzolar-*S*-methyl (ACTIGARD®).

Tank mix	Control (%)	E_e	E/E_e
Reynoutria extract + acibenzolar-*S*-methyl	84.2	51.5	1.6

Table 13. Disease severity and percentage control of MBI-106 (REGALIA® ME) and mefenoxam(RIDOMIL GOLD® SL)

Treatment	Dilution/Rate	Severity (%)	Control (%)
Untreated control	N/A	87.0ab	0.0
mefenoxam	150 ug/ml	44.8de	48.5
mefenoxam	75 ug/ml	54.2cd	37.7
mefenoxam	37.5 ug/ml	89.3a	-2.6
Reynoutria extract	400×	68.0bc	21.8
Reynoutria extract + mefenoxam	400× 150 ug/ml	14.5f	83.3
Reynoutria extract + mefenoxam	400× 75 ug/ml	28.6ef	67.1
Reynoutria extract + mefenoxam	400× 37.5 ug/ml	59.2cd	32.0

Table 14. The expected efficacy (E_e) of each product combination, actual control efficacy (E), and detected synergism (E/E_e>1.0) between MBI-106 (REGALIA® ME) and mefenoxam (RIDOMIL GOLD® SL).

Tank mix	Dilution/Rate	Control (%)	E_e	E/E_e
Reynoutria extract + mefenoxam	400× 150 ug/ml	83.3	59.7	1.4
Reynoutria extract + mefenoxam	400× 75 ug/ml	67.1	51.3	1.3
Reynoutria extract + mefenoxam	400× 37.5 ug/ml	32.0	19.7	1.6

Table 15. Emergence of soybean seedling and control of damping off by MBI-106 (ethanol extract) and azoxystrobin (QUADRIS®).

Treatment	Rate	Emergence (%)		Control (%)
Non-inoculated control	N/A	90.1	a	N/A
Inoculated control	N/A	4.9	c	0.0
Inoculated Reynoutria extract	0.02117g/kg	11.1	c	6.2
Inoculated Reynoutria extract + azoxystrobin	0.02117g/kg 0.0298g/kg	92.6	a	87.7
Inoculated azoxystrobin	0.0298g/kg	86.4	a	81.5

Table 16. The expected efficacy (E_e) of each product combination, actual control efficacy (E), and detected synergism (E/E_e>1.0) between MBI-106 (ethanol extract) and azoxystrobin (QUADRIS®).

Tank mix	Rate	Control (%)	E_e	E/E_e
Reynoutria extract + azoxystrobin	0.02117g/kg 0.0298g/kg	87.7	82.6	1.1

Table 17. Emergence of soybean seedlings and control of damping off by MBI-106 (*Reynoutria* ethanol extract) and fludioxonil (SCHOLAR®).

Treatment	Rate	Emergence (%)		Bio mass (g)		Control (%)
Inoculated control	N/A	7.4	c	0.8	c	0.0
Inoculated Reynoutria extract	0.635g/kg	9.9	c	1.4	c	2.5
Inoculated Reynoutria extract	0.03175g/kg	16.0	c	2.9	c	8.6
Inoculated Reynoutria extract + fludioxonil	0.635g/kg 0.0596g ai/kg	61.8	a	17.0	a	54.4
Inoculated Reynoutria extract + fludioxonil	0.03175g/kg 0.0596g ai/kg	50.6	a	12.5	b	43.2
Inoculated fludioxonil	0.0596g ai/kg	35.8	b	8.7	b	28.4

Table 18. The expected efficacy (E_e) of each product combination, actual control efficacy (E), and detected synergism ($E/E_e>1.0$) between MBI-106 (ethanol extract) and fludioxonil (SCHOLAR®).

Tank mix	Rate	Control (%)	E_e	E/E_e
Inoculated Reynoutria extract + fludioxonil	0.635g/kg 0.0596g ai/kg	54.4	30.2	1.8
Inoculated Reynoutria extract + fludioxonil	0.03175g/kg 0.0596g ai/kg	43.2	34.6	1.2

Table 19. Disease severity and percentage control of *R. sachalinensis* and propiconazole (PROPIMAX®) when used alone or in tank mix in controlling cucumber powdery mildew.

Treatment	Dilution/Rate	Severity (%)	Control (%)
Untreated control	N/A	98.8 a	0.0
propiconazole	1.0 ug/ml	97.5 a	1.3
Reynoutria extract	2500×	61.3 b	38.0
Reynoutria + propiconazole	2500× 1.0 ug/ml	51.3 c	48.1

Table 20. The expected efficacy (E_e) of each product combination, control efficacy (E), and the synergistic effect ($E/E_e > 1.0$) between MBI-106 (REGALIA® ME) and propiconazole (PROPIMAX®).

Tank mix	Dilution/Rate	control (%)	E^e	E/e_e
Reynoutria extract + propiconazole	2500× 1.0 ug/ml	48.1	38.8	1.2

Table 21. Disease severity and percentage control of *R. sachalinensis* and azoxystrobin (QUADRIS®) when used alone or in tank mix in controlling cucumber powdery mildew (

Treatment	Dilution/Rate	Severity%	Control (%)
Untreated control	N/A	80.0 a	13.5
azoxystrobin	0.5 ug/ml	35.0 cd	62.2
azoxystrobin	0.25 ug/ml	80.0 a	13.5
azoxystrobin	0.1 ug/ml	72.5 abc	21.6
Reynoutria extract	2000×	75.0 ab	18.9
Reynoutria extract + azoxystrobin	2000× 0.5 ug/ml	11.3 d	87.8
Reynoutria extract + azoxystrobin	2000× 0.25 ug/ml	41.3 abcd	55.4
Reynoutria extract + azoxystrobin	2000× 0.1 ug/ml	36.5 bcd	60.5

Table 22. The expected efficacy (E_e) of each product combination, control efficacy (E), and the synergistic effect ($E/E_e > 1.0$) between MBI-106 (Regalia® SC) and azoxystrobin (Quadris®) (Test II).

Tank mix	Dilution/Rate	Control (%)	E_e	E/E_e
Reynoutria extract + azoxystrobin	2000× 0.5 ug/ml	87.8	69.3	1.3
Reynoutria extract + azoxystrobin	2000× 0.25ug/ml	55.4	29.9	1.9
Reynoutria extract + azoxystrobin	2000× 0.1 ug/ml	60.5	36.4	1.7

Literature cited:

Bardin, M., J. Fargues, et al. (2008). "Compatibility between biopesticides used to control grey mold, powdery mildew and whitefly on tomato." Biological Control **46**: 476-483.

Bartlett, D. W., J. M. Clough, et al. (2002). "649-662." Pest Management Science **58**: 649-662.

Belanger, R. R. and M. Benyagoub (1997). "Challenges and prospoects for integrated control of powdery mildews in the greenhouse." Canadian Journal of Plant Pathology **19**: 310-314.

Bokshi, A. I., J. Jobling, et al. (2008). "A single application of Milsana followed by Bion assists in the control of powdery mildew in cucumber and helps overcome yield losses." Journal of Horticultural Science and Biotechnology **83**: 701-706.

Braun, U., T. A. Cook, et al. (2002). The taxonomy of the powdery mildew fungi. The powdery mildews: a comprehensive treatise. R. R. Belanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver. St. Paul, MN, APS Press: 13-55.

Burpee, L. and R. Latin (2008). "Reassessment of fungicide synergism for control of dollar spot." Plant Disease **92**: 601-606.

Daayf, F., A. Schmitt, et al. (1995). "The effects of plant extracts of Reynoutria sachalinensis on powdery mildew development and leaf physiology of long English cucumber." Plant Disease **79**: 577-580.

De Waard, M. A. (1996). "Synergism and antagonism in fungicide mixtures containing sterol demethylation inhibitors." Phytopathology **86**: 1280-1283.

Durrant, W. E. and X. Dong (2004). "Systemic acquired resistance." Annual Review in Phytopathology **42**: 185-209.

Fraaije, B., J. A. Lucas, et al. (2003). QoI resistance development in populations of cereal pathogens in the UK. BCPC Internatrional Congress - Crop Science and Technology, Alton, Hants, UK, pp. 689-694.

Gandhi, N. R., V. P. Skebba, et al. (2007). Antimycotic rhamnolipid compositions and related methods of use, US patent application number 20070191292. USPTO. USA: 27 p.

Gisi, U. (1996). "Synergistic interactions of fungicides in mixtures " Phytopathology **86**: 1273-1279.

Hafez, M. B., A. Schmitt, et al. (1999). "The side-effects of plant extracts and metabolites of *Reynoutria sachalinensis* (F. Schmidt) Nakai and conventional fungicides on the beneficial organism *Trichogramma cacoeciae* Marchal (Hym., *Trichogrammatidae*)." Journal of Applied Entomology **123**: 363-368.

Holb, I. J. and G. Schnabel (2008). "The benefits of combining elemental sulfur with a DMI fungicide to control *Monilinia fructicola* isolates resistant to propiconazole." Pest Management Science **64**: 156-164.

Horst, R. K., S. O. Kawamoto, et al. (1992). "Effect of sodium bicarbonate and oils on the control of powdery mildew and black spot on roses." Plant Disease **76**: 247-251.

Hwang, S.F., H. Wang, et al. (2006). "Effect of seed treatment and root pathogens on seedling establishment and yield of alfalfa, birdfoot trefoil and sweetclover." Plant Pathology Journal **5**:322-328.

James, W. C. (1971). A manual assessment keys for plant diseases. St. Paul, MN, American Phytopathological Society.

Karaoglanidis, G. S. and D. A. Karadimos (2006). "Efficacy of strobilurins and mixtures with DM fungicides in controlling powdery mildew in field-grown sugar beet." Crop Protection **25**: 977-983.

Konstantinidou-Doltsinis, S., E. Markellou, et al. (2007). "Control of powdery mildew of grape in Greece using Sporodex L and Milsana." Journal of Plant Diseases and Protection **114**: 256-262.

Limpel, L. E., P. H. Schuldt, et al. (1962). N.E. Weed Control Conference.

May, R. M. (1985). "Evolution of pesticide resistance." Nature **315**: 12-13.

McGrath, M. T. (2001). "Fungicide resistance in cucurbit powdery mildew: Experiences and challenges." Plant Disease **85**: 236-245.

Reuveni, M. (2001). "Improved control of powdery mildew (*Sphaerotheca pannosa*)of nectarines in Israel using strobilurin and polyoxin B fungicides; mixtures with sulfur; and early bloom applications." Crop Protection **20**: 663-668.

Richer, D. (1987). "Synergism - a patent view." Pesticide Science **19**: 309-315.

Ross, A. F. (1961). "Systemic acquired resistance induced by localized virus infections in plants " Virology **14**: 340-358.

Samoucha, Y. and Y. Cohen (1984). "Synergy between metalaxyl and macozeb in controlling downy mildew in cucumbers." Phytopathology **74**: 1434-1437.

Schmitt, A. (2002). "Induced responses by plant extracts from *Reynoutria sachalinensis*: a case study." Bull. IOBC/WPRS **25**: 83-89.

Schmitt, A., S. Kunz, et al. (2002). Use of *Reynoutria sachalinensis* plant extracts, clay preparations and *Brevibacillus brevis* against fungal diseases of grape berries. Fordergemeinschaft Okologisher Obstbau e.V.(FOKO) and der Staatlichen Lehr- und Versuchsanstalt fur Wein- und Obstbau (LvWO) Weinsberg. 10th International conference on cultication technique and phytopathological problems in organic fruit-growing and viticulture; presentations at the meeting from 04-07.02.2002
Weinsberg, Germany, pp. 146-151.

Schmitt, A. and B. Seddon (2005). Biocontrol of plant pathogens with microbial BCAs and plant extracts - advantages and disadvantages of single and combined use. Modern fungicides and antifungal compounds IV. Proceedings of the 14th International Reinhardsbrunn Symposium 2004, BCPC, Atlon, UK, pp. 205-225.

Schnabel, G., B. K. Bryson, et al. (2004). "Reduced sensitivity in *Monilinia fructicola* to propiconazole in Georgia and implcations for disease management " Plant Disease **88**: 1000-1004.

Su, H., A. H. C. van Bruggen, et al. (2004). "Sporulation of *Bremia lactucae* affected by temperature, relative humidity, and wind in controlled conditions". Phytopathology **94**:396-401

Tuttle McGrath, M. (2003). "Occurrence of strobilurin resistance and impact on managing powdery mildew on cucurbits." Cornell University; Vegetable MD Online Retrieved July 19, 2009, 2009, from http://vegetablemdonline.ppath.cornell.edu/NewsArticles/Cuc_Strob.htm.

Tuttle McGrath, M. (2006). "Guidelines for managing cucurbit powdery mildew in 2006." Cornell University, Vegetable MD Online Retrieved July 19, 2009, 2009, from http://vegetablemdonline.ppath.cornell.edu/NewsArticles/Cuc_PM_2006.html.

Van den Bosch, F. and C. A. Gilligan (2008). "Models of fungicide resistance dynamics." Annual Review in Phytopathology **46**: 123-147.

Vechet, L., L. Burketova, et al. (2009). "A comparative study of the efficiency of several sources of induced resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici*) in wheat under field conditions." Crop Protection **28**: 151-154.

Walters, D., D. Walsh, et al. (2005). "Induced resistance for plant disease control: maximizing the efficacy of resistance elicitors." Phytopathology **85**: 1368-1373.

Wurms, K., C. Labbe, et al. (1999). "Effects of Milsana and Benzothiadiazole on the ultrastructure of powdery mildew haustoria in cucumber." Phytopathology **89**: 728-736.

Wyenandt, C. A., M. T. McGrath, et al. (2009). "Fungicide resistance management guidelines for cucurbit downy and powdery mildew control in the mid-Atlantic and Northeast regions of the US." Phytopathology **99** (2009 APS Annual Meeting Abtsracts of Presentations): S144-S144.

WHAT IS CLAIMED IS:

1. A synergistic mixture for use in modulating fungal infection comprising:
   (a) a *Reynoutria sachalinensis* extract, wherein said extract contains physcion and
   (b) an azoxystrobin.

2. The synergistic mixture according to claim 1, wherein synergism of said synergistic mixture is measured by determining E/Ee, wherein said E/Ee is greater than 1.3.

3. The synergistic mixture according to claim 1 or 2, wherein the mixture further comprises chrysophanol.

4. The synergistic mixture according to any one of claims 1-3, wherein the fungal infection is a powdery mildew infection.

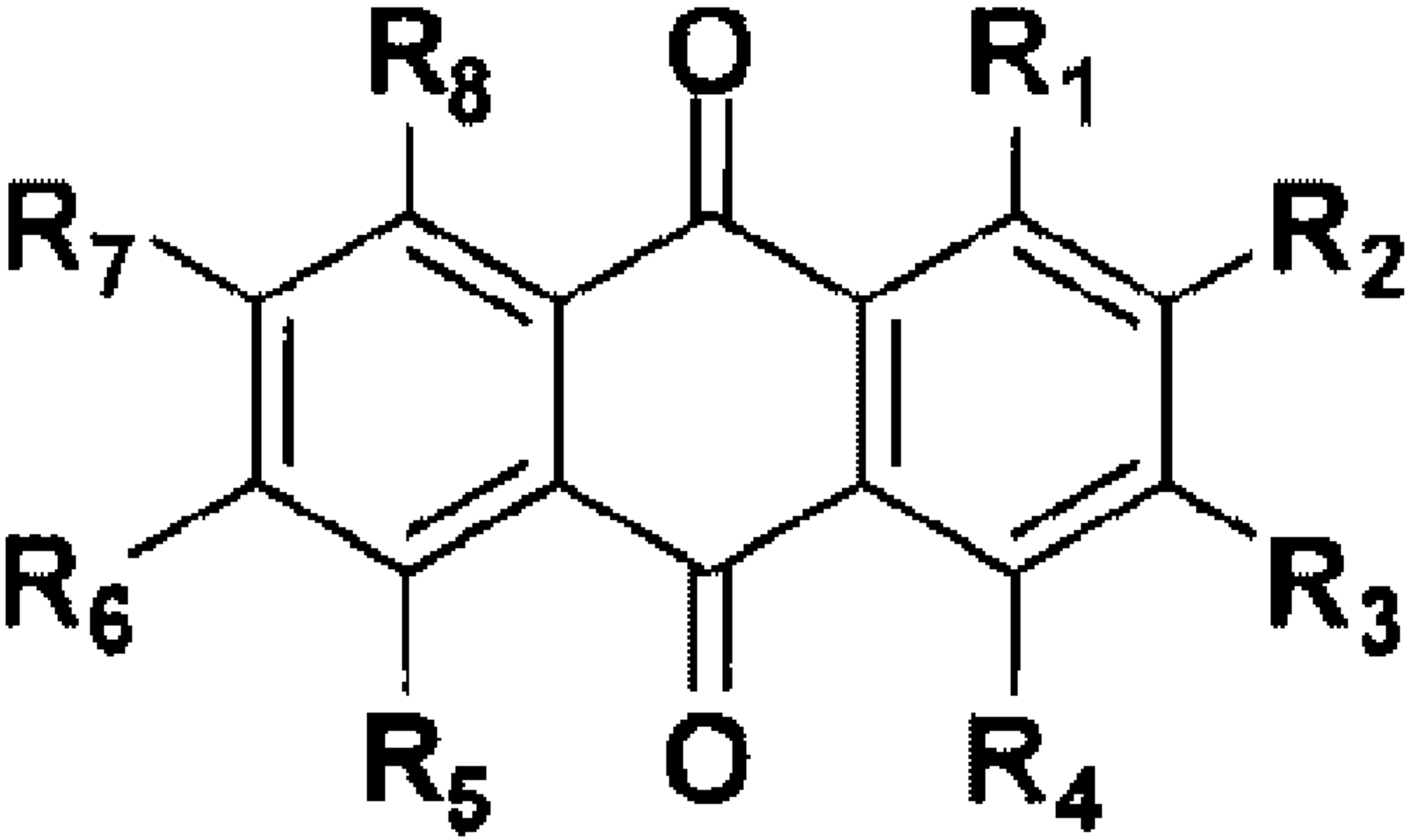
5. The synergistic mixture according to any one of claims 1-3, wherein the fungal infection is a *Rhizoctonia solani* infection.

6. The synergistic mixture according to any one of claims 1-5, wherein the mixture further comprises emodin.

7. A method for modulating fungal infection in a plant comprising applying to the plant and/or seeds thereof and/or substrate used for growing said plant an amount of the mixture as defined in any one of claims 1-6, effective to modulate said fungal infection.

8. The method according to claim 7, wherein the fungal infection is a powdery mildew infection.

9. The method according to claim 7, wherein the fungal infection is a *Rhizoctonia solani* infection.



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