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(71) Applicant: **SYNGENTA CROP PROTECTION AG** [CH/CH]; Rosentalstrasse 67, 4058 Basel (CH).

(72) Inventors: **SCREPANTI, Claudio**; Syngenta Crop Protection AG, Schaffhauserstrasse, 4332 Stein (CH). **BERGNA, Alessandro**; Syngenta Crop Protection AG, Schaffhauserstrasse, 4332 Stein (CH).

(74) Agent: **SYNGENTA IP**; Rosentalstrasse 67, 4058 Basel (CH).

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(54) Title: USE OF RIBOFLAVIN AS A SEED TREATMENT

(57) Abstract: A method of controlling, limiting or preventing an infestation of a crop plant by a phytopathogenic fungi of the genus Fusarium by contacting plant propagation material, prior to or during sowing of the crop plant, with a composition comprising riboflavin.

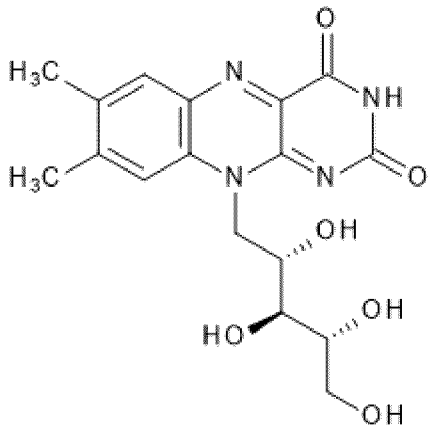
WO 2024/251904 A1

USE OF RIBOFLAVIN AS A SEED TREATMENT

The present invention relates to the use of riboflavin as a seed treatment for controlling certain soil-borne diseases caused by phytopathogenic fungi, in particular fungi of the genus Fusarium (such as Fusarium wilt), and to a seed treatment composition comprising riboflavin for use in crop protection.

Fusarium is a large genus of filamentous fungi comprising *Fusarium oxysporum*, *Fusarium solani*, *Fusarium fujikuroi*, and *Fusarium graminearum*. Fusarium species are commonly found in soil, creating a disease threat to commercially-important agricultural plant crops, especially in the form of soil-borne diseases caused by *Fusarium oxysporum*, which may be very difficult to control.

Riboflavin (CAS no.: 83-88-5, also known as vitamin B2) is a water-soluble vitamin commonly found in food and also used as a human dietary supplement. Its uses in agriculture for crop protection purposes has hitherto been limited.



Riboflavin

It has now been surprisingly found that riboflavin when used as a seed treatment can be effective in controlling disease caused by Fusarium species.

According to the present invention, there is provided a method of controlling, limiting or preventing an infestation of a crop plant by a phytopathogenic fungi of the genus *Fusarium* by contacting plant propagation material, prior to or during sowing of the crop plant, with a composition comprising riboflavin.

According to a second aspect of the present invention, there is provided a seed treatment composition comprising riboflavin, and further comprising an agrochemically acceptable diluent or carrier.

According to a third aspect of the present invention, there is provided a crop plant seed treated with a composition according to the invention.

According to a fourth aspect of the present invention, there is provided the use of riboflavin as a seed treatment ingredient for the control of a phytopathogenic fungi of the genus *Fusarium* in a crop plant.

Surprisingly, it has been found that riboflavin has, for practical purposes, an advantageous level of biological activity for protecting plants against disease caused by phytopathogenic fungi of the genus *Fusarium* when used as a seer treatment.

By biological activity, it is meant that the compositions of the invention are capable of killing or controlling the growth of phytopathogenic fungi of the genus *Fusarium*, and/or reducing damage to plants caused by the phytopathogenic fungi of the genus *Fusarium*.

Preferably, the phytopathogenic fungi is *Fusarium oxysporum.*

Preferably, the method of the invention, further comprises the step of sowing the seed in a soil substrate susceptible to, or with, the presence of phytopathogenic fungi of the genus *Fusarium*.

The compositions used according to the present invention may further comprise a pesticidal active ingredient which may be an insecticide, a fungicide, a herbicide, a synergist, a plant growth regulator, a nematicide, a plant nutrient, a plant fertilizer, or a mixture thereof.

Riboflavin may be applied to a plant seed as a seed treatment in a composition in the absence of a pesticidal active ingredient. However, it may also be applied in combination with pesticidal (eg, fungicidal, insecticidal or nematicidal) active ingredients. This may include pesticidal compositions comprising riboflavin (represented by "(I)") and a pesticidal active ingredient as follows:

acibenzolar-s-methyl + (I), metalaxyl-M (mefenoxam) + (I), metalaxyl + (I), picarbutrazox + (I), oxathiapiprolin + (I), ethaboxam + (I), fludioxonil + (I), azoxystrobin + (I), thiabendazole + (I), difenoconazole + (I), tebuconazole + (I), ipconazole + (I), mefentrifluconazole + (I), prothioconazole + (I), triticonazole + (I), sedaxane + (I), inpyrfluxam + (I), penflufen + (I), fluopyram + (I), fluxapyroxad + (I), pydiflumetofen + (I), cyclobutrifluram + (I), thiamethoxam + (I), isoflucypram + (I), clothianidin + (I), *Bacillus amyloliquefaciens* + (I), benzovindiflupyr + (I), cymoxanil + (I), phosphite (phosphonate) + (I), silthiofam + (I), pyraclostrobin + (I), isotianil + (I), tolprocarb + (I), quinofumelin + (I), ipflufenoquin + (I), metyltetraprole + (I), florylpicoxamid + (I), fenpicoxamid + (I), fluoxytioconazole + (I), fluindapyr + (I), fluoxapiprolin + (I), broflanilide + (I), imidacloprid + (I), acetamiprid + (I), cyantraniliprole + (I), chlorantraniliprole + (I), tetraniliprole + (I), fluxamethamide + (I), penthiopyrad + (I), mandipropamid + (I), dimpropyridaz + (I), flupyrimin + (I), triflumezopyrim + (I), oxazosulfyl + (I), dichlobentiazox + (I), benzpirimoxan + (I), hymexazol + (I), pyrapropoyne + (I), cyclaniliprole + (I), mandestrobin + (I), copper + (I), lambda-cyhalothrin + (I), phenamacril + (I), abamectin + (I), tefluthrin + (I), picoxystrobin + (I), spiropidion + (I), oligosaccharins + (I), *Melaleuca alternifolia* + (I), fosthiazate + (I), fenamifos + (I), oxamyl + (I), fluensulfone + (I), fluazaindolizine + (I), spirotetramat + (I), spidoxamat + (I), fluazinam + (I), indazapyroxamet + (I), thiophanate methyl + (I), zoxamide + (I), fluoxastrobin + (I), pirimifos + (I), tetrachlorantraniliprole + (I), bifenthrin and emamectin benzoate + (I).

In any case, the weight ratio of riboflavin and a fungicidal, insecticidal or nematicidal active ingredient is generally between 1000:1 and 1:100, more preferably between 500:1 and 1:100, for example, between 250:1 and 1:66, between 50:1 to 1:50, between 33:1 and 1:5, between 8:1 and 1:3, and between 2:1 to 1:2 (including 1:1).

In certain embodiments of the method and composition of the invention, the composition may further comprise a fungicidal, insecticidal or nematicidal active ingredient at a weight ratio range to riboflavin of 1:50 to 50:1, and preferably 1:10 to 10:1.

Crops of useful plants in which the use and method of the invention may be applied include perennial and annual crops, such as:

berry plants, eg, blackberries, blueberries, cranberries, raspberries and strawberries.

cereals, eg, barley, maize (corn), millet, oats, rice, rye, sorghum triticale and wheat.

fibre plants, eg, cotton, flax, hemp, jute and sisal.

field crops, eg, sugar and fodder beet, coffee, hops, mustard, oilseed rape (canola), poppy, sugar cane, sunflower, tea and tobacco.

fruit trees, eg, apple, apricot, avocado, banana, cherry, citrus, nectarine, peach, pear and plum.

grasses, eg, Bermuda grass, bluegrass, bentgrass, centipede grass, fescue, ryegrass, St. Augustine grass and Zoysia grass.

herbs, eg, basil, borage, chives, coriander, lavender, lovage, mint, oregano, parsley, rosemary, sage and thyme.

legumes, eg, beans, lentils, peas and soybeans.

nuts, eg, almond, cashew, ground nut, hazelnut, peanut, pecan, pistachio and walnut.

palms, eg, oil palm.

ornamentals, eg, flowers, shrubs and trees.

other trees, eg, cacao, coconut, olive and rubber.

vegetables, eg, asparagus, aubergine, broccoli, cabbage, carrot, cucumber, garlic, lettuce, marrow, melon, okra, onion, pepper, chilli, potato, pumpkin, rhubarb, spinach and tomato. and

vines, eg, grapes.

Preferably, the method and composition of the invention relate to treatment application to a plant propagation material (including a seed) of a crop of a useful plant selected from cotton, cereals, eg, barley, maize (corn), millet, oats, rice, rye, sorghum triticale, wheat and soybean, and more preferably to seeds of a crop plant selected from cotton, maize (corn), wheat or soybean.

In accordance with the the present invention, propagation material of a plant can be understood to denote all the generative parts of the plant, such as seeds, which can be used for the multiplication of the latter including vegetative plant material such as cuttings. There may be mentioned, as plant propagation material, seeds (in the strict sense), roots, fruits, tubers, bulbs, rhizomes, parts of plants. The plant propagation material can be treated in accordance with the invention before the material is sown or planted. Alternatively, the plant propagation material may be treated during sowing or planting. Additionally, the riboflavin may be applied to the previously treated propagation material before or during

its planting. Riboflavin may be applied during the sowing of the seed or may also be used to plant propagation material derived from plants grown in a green house and/or during transplantation. More preferably, the plant propagation material is plant seeds.

The seed treatment can occur to an unsown seed, and the term "unsown seed" is meant to include seed at any period between the harvest of the seed and the sowing of the seed in the ground for the purpose of germination and growth of the plant. Treatment to an unsown seed is not meant to include those practices in which a pesticide is applied to the soil but would include any application practice that would target the seed during the sowing/planting process.

The treated plant propagation material of the present invention can be treated in the same manner as conventional plant propagation material. The treated propagation material can be stored, handled, sown and tilled in the same manner as any other pesticide treated material.

In the present invention, the application rate in terms of the weight ratio of riboflavin to seed (or other plant propagation material) is 10 mg to 5 g of riboflavin per kg of seeds, 50 mg to 2 g of riboflavin per kg of seeds, and preferably 100 mg to 1 g of riboflavin per kg of seeds.

In practice, the agrochemical compositions used in accordance with the invention are applied as a formulation containing the various adjuvants and carriers known to or used in the industry.

According to the present invention, riboflavin and any other active ingredient(s) can be advantageously in the form of a soluble concentrate (SL) or a flowable concentrate for seed treatment (FS) or a suspension concentrate (SC) and can be more preferably a seed treatment slurry to be applied onto seeds. Slurries for seed treatment applications are well-known in the art. For example, a slurry can contain the active ingredient(s), in the form of a commercially available product or not, mixed with water and optionally with at least one polymer as described in the present invention, to optimize adhesion around the plant propagation material.

In the method of the present invention, riboflavin and any accompanying pestcidial active ingredient(s) can be generally in the form of a composition or formulation containing other customary formulation adjuvants because it allows, for example, less burdensome handling and application.

Riboflavin and any accompanying pestcidial active ingredient(s) can be applied to the plant propagation material from different compositions respectively, or the ingredients can be gathered in the same composition (ready-mix composition). The composition may contain from about 0.001% to about 99% by weight of the active ingredient(s) over the total weight of the composition. Suitably, the composition contains from about 0.001% to about 50% by weight active ingredient(s) over the total weight of the composition. More suitably, the composition contains from about 0.001% to about 10% by weight active ingredient(s) over the total weight of the composition. More suitably, the composition contains from about 0.001 % to about 1% by weight active ingredient(s) over the total weight of the composition. If the formulation is in the form of a concentrate, requiring dilution with water before use, it will contain a higher amount of active ingredients than a composition that is ready to use without dilution.

Riboflavin and any accompanying pestcidial active ingredient(s) used according to the invention can be used as pesticidal agents in unmodified form, but they are generally formulated into compositions in various ways using formulation adjuvants, such as liquid carriers (or solvents), solid carriers, and surface-active substances. Such formulations can either be used directly or diluted prior to use. The

dilutions can be made, for example, with water, liquid fertilizers, micronutrients, biological organisms, oil and/or solvents.

The formulations can be prepared, eg, by mixing riboflavin and any accompanying pestcidial active ingredient(s) with formulation adjuvants in order to obtain compositions in the form of finely divided solids, granules, solutions, dispersions or emulsions. The ingredients can also be formulated with other adjuvants, such as finely divided solids, mineral oils, oils of vegetable or animal origin, modified oils of vegetable or animal origin, organic solvents, water, surface-active substances, or any mixture thereof.

The formulation adjuvants that are suitable for the preparation of the compositions used according to the invention are known *per se*.

As liquid carriers there may be used: water, toluene, xylene, petroleum ether, vegetable oils, acetone, methyl ethyl ketone, cyclohexanone, acid anhydrides, acetonitrile, acetophenone, amyl acetate, 2-butanone, butylene carbonate, chlorobenzene, cyclohexane, cyclohexanol, alkyl esters of acetic acid, diacetone alcohol, 1,2-dichloropropane, diethanolamine, p-diethylbenzene, diethylene glycol, diethylene glycol abietate, diethylene glycol butyl ether, diethylene glycol ethyl ether, diethylene glycol methyl ether, N,N-dimethylformamide, dimethyl sulfoxide, 1,4-dioxane, dipropylene glycol, dipropylene glycol methyl ether, dipropylene glycol dibenzoate, diproxitol, alkylpyrrolidone, ethyl acetate, 2-ethylhexanol, ethylene carbonate, 1,1,1-trichloroethane, 2-heptanone, alpha-pinene, d-limonene, ethyl lactate, ethylene glycol, ethylene glycol butyl ether, ethylene glycol methyl ether, gamma-butyrolactone, glycerol, glycerol acetate, glycerol diacetate, glycerol triacetate, hexadecane, hexylene glycol, isoamyl acetate, isobornyl acetate, isooctane, isophorone, isopropylbenzene, isopropyl myristate, lactic acid, laurylamine, mesityl oxide, methoxypropanol, methyl isoamyl ketone, methyl isobutyl ketone, methyl laurate, methyl octanoate, methyl oleate, methylene chloride, m-xylene, n-hexane, n-octylamine, octadecanoic acid, octylamine acetate, oleic acid, oleylamine, o-xylene, phenol, polyethylene glycol, propionic acid, propyl lactate, propylene carbonate, propylene glycol, propylene glycol methyl ether, p-xylene, toluene, triethyl phosphate, triethylene glycol, xylenesulfonic acid, paraffin, mineral oil, trichloroethylene, perchloroethylene, ethyl acetate, amyl acetate, butyl acetate, propylene glycol methyl ether, diethylene glycol methyl ether, methanol, ethanol, isopropanol, and alcohols of higher molecular weight, such as amyl alcohol, tetrahydrofurfuryl alcohol, hexanol, octanol, ethylene glycol, propylene glycol, glycerol, N-methyl-2-pyrrolidone and the like.

The solid carrier can be a natural or synthetic solid material that is insoluble in water. This carrier is generally inert and acceptable in agriculture, especially on the treated seed or other propagation material. It can be chosen, for example, from clay, diatomaceous earth, natural or synthetic silicates, titanium dioxide, magnesium silicate, aluminum silicate, talc, pyrophyllite clay, silica, attapulgite clay, kieselguhr, chalk, limestone, calcium carbonate, calcium montmorillonite, bentonite clay, Fuller's earth, cottonseed husks, wheat flour, soybean flour, pumice, wood flour, ground walnut shells, lignin, and the like.

A large number of surface-active substances can advantageously be used in both solid and liquid formulations, especially in those formulations which can be diluted with a carrier prior to use. Surface-active substances may be anionic, cationic, non-ionic or polymeric, and they can be used as emulsifiers, wetting agents or suspending agents or for other purposes. Typical surface-active substances include, for example, salts of alkyl sulfates, such as diethanolammonium lauryl sulfate; salts of

alkylarylsulfonates, such as calcium dodecylbenzenesulfonate; alkylphenol/alkylene oxide addition products, such as nonylphenol ethoxylate; alcohol/alkylene oxide addition products, such as tridecylalcohol ethoxylate; soaps, such as sodium stearate; salts of alkylnaphthalenesulfonates, such as sodium dibutylnaphthalenesulfonate; dialkyl esters of sulfosuccinate salts, such as sodium di(2-ethylhexyl)sulfosuccinate; sorbitol esters, such as sorbitol oleate; quaternary amines, such as lauryltrimethylammonium chloride, polyethylene glycol esters of fatty acids, such as polyethylene glycol stearate; block copolymers of ethylene oxide and propylene oxide; and salts of mono- and di-alkylphosphate esters; and also further substances described e.g. in McCutcheon's Detergents and Emulsifiers Annual, MC Publishing Corp., Ridgewood New Jersey (1981).

Further adjuvants that can be used in pesticidal formulations include crystallisation inhibitors, viscosity modifiers, suspending agents, dyes, anti-oxidants, foaming agents, light absorbers, mixing auxiliaries, antifoams, complexing agents, neutralising or pH-modifying substances and buffers, corrosion inhibitors, fragrances, wetting agents, take-up enhancers, micronutrients, plasticisers, glidants, lubricants, dispersants, thickeners, antifreezes, microbicides, and liquid and solid fertilisers.

The compositions used according to the present invention generally comprise from 1 to 99.9 % by weight of a formulation adjuvant over the total weight of the composition, which preferably includes from 0 to 25 % by weight of a surface-active substance over the total weight of the composition. Whereas, commercial products may preferably be formulated as concentrates, the end user will normally employ dilute formulations.

Even distribution of the composition of the invention and adherence thereof to the propagation material is desired during the treatment process, this is particularly preferable when the propagation material is a seed. The treatment could vary from a thin film of the formulation containing the composition of the invention on a plant propagation material, such as a seed, where the original size and/or shape are recognizable to a thick film (such as a coating or pelleting with many layers of different materials (such as carriers, for example, clays; different formulations, such as of active ingredients; polymers; and colourants)) where the original shape and/or size of the seed is no longer recognisable.

More particularly, said thin film can be a film-forming polymer well-known in the art. The composition can include at least one polymer selected from water-soluble and water-dispersible film-forming polymers. Suitable polymers have an average molecular weight of at least about 1,000 up to about 100,000; more specifically at least about 5,000, up to about 100,000. The aqueous compositions generally contain from about 0.5% to about 10% by weight of the composition of polymer (b). In a specific embodiment, the compositions contain from about 1.0% up to about 5% by weight of a film-forming polymer. Suitable polymers are selected from alkyleneoxide random and block copolymers such as ethylene oxide-propylene oxide block copolymers (EO/PO block copolymers) including both EO-PO-EO and PO-EO-PO block copolymers; ethylene oxide-butylene oxide random and block copolymers; C2-6 alkyl adducts of ethylene oxide-propylene oxide random and block copolymers; C2-6 alkyl adducts of ethylene oxide-butylene oxide random and block copolymers; polyoxyethylene-polyoxypropylene monoalkylethers such as methyl ether, ethyl ether, propyl ether, butyl ether or mixture thereof; vinylacetate/vinylpyrrolidone copolymers; alkylated vinylpyrrolidone copolymers; polyvinylpyrrolidone; polyalkyleneglycol including the polypropylene glycols and polyethylene glycols; and any mixture thereof. Specific examples of suitable polymers include Pluronic P103 (BASF) (EO-PO-EO block

copolymer), Pluronic P65 (BASF) (EO-PO-EO block copolymer), Pluronic P108 (BASF) (EO-PO-EO block copolymer), Vinamul 18160 (National Starch) (polyvinylacetate), Agrimer 30 (ISP) (polyvinylpyrrolidone), Agrimer VA7w (ISP) (vinyl acetate/vinylpyrrolidone copolymer), Agrimer AL 10 (ISP) (alkylated vinylpyrrolidone copolymer), PEG 400 (Uniqema) (polyethylene glycol), Pluronic R 25R2 (BASF) (PO-EO-PO block copolymer), Pluronic R 31R1 (BASF) (PO-EO-PO block copolymer) and Witconol NS 500LQ (Witco) (butanol PO-EO copolymer).

Accordingly, riboflavin and any accompanying pestcidial active ingredient(s), and more preferably the composition(s) comprising these ingredient(s), can preferably adhere to the propagation material, such as a seed.

Although it is believed that the present method can be applied to a seed in any physiological state, it is preferred that the seed be in a sufficiently durable state that it incurs no damage during the treatment process. Typically, the seed would be a seed that had been harvested from the field; removed from the plant; and separated from any cob, stalk, outer husk, and surrounding pulp or other non-seed plant material. The seed would preferably also be biologically stable to the extent that the treatment would cause no biological damage to the seed. It is believed that the treatment can be applied to the seed at any time between harvest of the seed and sowing of the seed or during the sowing process (seed directed applications).

The composition used according to the present invention can be applied to seeds using conventional treating techniques and machines, such as fluidized bed techniques, the roller mill method, rotostatic seed treaters, and drum coaters. Other methods, such as spouted beds may also be useful. The seeds may be pre-sized before coating. After coating, the seeds are typically dried and then transferred to a sizing machine for sizing. Such procedures are known in the art and are set out in more detail below.

The composition used in the method according to the present invention can be called a dressing composition, which relates to a liquid composition useful for covering and/or wetting a plant propagation material, and more preferably a seed, at least in part or in totality.

The composition according to the present invention is particularly suited for dressing applications on plant propagation material, especially on seeds. The techniques of seed treatment application are well known to those skilled in the art, and they may be used readily in the context of the present invention. The compositions of the invention may be applied to the seed as slurry or a soak. There also may be mentioned, e.g., film coating or encapsulation. The coating processes are well known in the art, and employ, for seeds, the techniques of film coating or encapsulation, or for the other multiplication products, the techniques of immersion.

One method of applying the composition according to the invention consists in spraying or wetting the plant propagation material with the aqueous liquid preparation or mixing the plant material with such liquid preparation. Also, before the application, the composition of the invention may be diluted with water by simple mixing at ambient temperature in order to prepare an on-farm seed treatment formulation.

Typically, a tank-mix formulation for seed treatment application comprises 0.25 % to 80 % by weight, especially 1 to 75 % by weight, of riboflavin and any accompanying pestcidial active ingredient(s), and 99.75 % to 20 % by weight, especially 99 % to 25 % by weight, of a solid or liquid

auxiliary (including, for example, a solvent such as water), where the auxiliaries can be a surfactant in an amount of 0 to 40 % by weight, especially 0.5 to 30 % by weight, based on the total weight of the tank-mix formulation.

Typically, a pre-mix formulation for seed treatment application comprises 0.5 to 99.9 % by weight, especially 1 to 95 % by weight, of riboflavin and any accompanying pestcidial active ingredient(s), and 99.5 to 0.1 % by weight, especially 99 to 5 % by weight, of a solid or liquid adjuvant (including, for example, a solvent such as water), where the auxiliaries can be a surfactant in an amount of 0 to 50 % by weight, especially 0.5 to 40 % by weight, based on the total weight of the pre-mix formulation.

Whereas commercial products will preferably be formulated as concentrates (e.g., pre-mix composition (formulation)), the end user will normally employ dilute formulations (e.g., tank mix composition).

EXAMPLES:

Methods

The fungicidal control effects of a riboflavin seed treatment have been observed via an experiment using cotton plants (*Gossypium hirsutum* cv. *Azahar*) and *Fusarium oxysporum*. To highlight the beneficial effect of the application of riboflavin as seed treatment, the study compared this application with untreated plants and with the application of riboflavin as soil mixture (study outline in Table 1 below).

Riboflavin is commercially available and was obtained from Sigma Aldrich.

Seeds of cotton plants were initially surface sterilized using a 10% sodium hypochlorite solution and subsequently coated with a water-based solution of riboflavin (RBF) at three different rates: 10 g_{RBF} / 100 kg_{seeds}, 40 g_{RBF} / 100 kg_{seeds}, 80 g_{RBF} / 100 kg_{seeds}.

For this experiment, 1.2 L plastic pots were used and the soil selected was an organic-matter rich top-soil based mixture (pH = 6.4; fertilized with osmocote Exact Mini slow-release fertilizer, 700 mL / pot).

The riboflavin soil mixture was prepared by mixing riboflavin (as solid powder) in pre-incubated soil. The resulting substrate was thereafter incubated in the dark for one week before sowing of cotton plant seeds. The concentration of riboflavin in the soil was of 37.6 ppm.

In order to induce *Fusarium* infestation, the soil of infested treatments (see study outline in Table 1) has been mixed with dried and grounded millet seeds (0.25% w/v) infested with *Fusarium oxysporum* f.sp. *vasinfectum*. Five seeds per pot have been planted at a depth of 2 cm and maintained in a greenhouse environment (temperature: 25°C, photoperiod: 14 h). The soil was kept well moist throughout the whole experiment.

The assessment of the severity of the fungal infestation was carried out 28 days after sowing with a visual inspection of disease symptoms on the aerial part of the plant. A score from 0 to 4, with 0 referring to “no symptoms” and 4 to “severe symptoms/plant death”, was assigned to each plant. At the same time, fresh shoot weight over the same period was recorded.

Table 1. Study design.

Treatment	*Fusarium* infestation	Riboflavin	Replicates
Untreated Control	No	-	6
RBF soil-mix	No	Drench 37.6 ppm	6
ST RBF-10	No	10 g $_{RBF}$ / 100 kg $_{seeds}$	6
ST RBF-40	No	40 g $_{RBF}$ / 100 kg $_{seeds}$	6
ST RBF-80	No	80 g $_{RBF}$ / 100 kg $_{seeds}$	6
Infested control	Yes: 0.25% w/v	-	6
Infested RBF soil-mix	Yes: 0.25% w/v	Drench 37.6 ppm	6
Infested ST RBF-10	Yes: 0.25% w/v	10 g $_{RBF}$ / 100 kg $_{seeds}$	6
Infested ST RBF-40	Yes: 0.25% w/v	40 g $_{RBF}$ / 100 kg $_{seeds}$	6
Infested ST RBF-80	Yes: 0.25% w/v	80 g $_{RBF}$ / 100 kg $_{seeds}$	6

The effect of a riboflavin seed treatment has been observed by both i) the reduction of the severity of *Fusarium*-related disease symptoms and ii) by the higher fresh weight of the plants.

The visual assessment of disease severity (score from 0 to 4) highlighted a significant reduction in the severity of *Fusarium*-related disease symptoms in the cotton plants generated by seeds treated with riboflavin. Conversely, plants treated with riboflavin soil-drench application did not show a consistent reduction of infestation-related symptoms.

More precisely, *Fusarium*-infested untreated plants had an average disease-severity score of 3.07 (standard error ± 0.31), while plants treated with riboflavin had a lower disease-severity scores of respectively: 1.70 (standard error ± 0.34) for the 10 g $_{RBF}$ / 100 kg $_{seeds}$ rate, 1.90 (standard error ± 0.33) for the middle rate, and 1.15 (standard error ± 0.34) for the higher rate (Figure 1a and Table 2). Moreover, seed treatment with riboflavin, at all tested rates, conferred greater control compared to the riboflavin soil-mix treatment. No disease symptoms were observed in non-infested plants.

The beneficial effect of riboflavin seed treatment against soil-borne diseases was also observed in the development of plant biomass and general vigor of the young seedlings. Seed treatment with riboflavin generated cotton plants with a higher shoots fresh weight in comparison with non-treated cotton plants infested with *Fusarium* (Table 2). The plant weight increment was respectively of: +41 % for the 10 g $_{RBF}$ / 100 kg $_{seeds}$ rate, + 49% for the 40 g $_{RBF}$ / 100 kg $_{seeds}$ rate, + 73 % for the 80 g $_{RBF}$ / 100 kg $_{seeds}$ rate. Finally, seed treatment with riboflavin, at all tested rates, resulted in plants with higher fresh weight compared to the plants treated with riboflavin as soil-mix treatment.

No fresh weight variation has been observed between riboflavin seed-treated and non-treated plants in absence of *Fusarium* infestation.

Table 2. Disease score data and shoot fresh weight data.

Treatment	Disease Score (0-4)		Shoot Fresh Weight (g)	
	Average	St. Error	Average	St. Error
Untreated Control	0.00	0.00	7.89	0.35
RBF soil-mix	0.00	0.00	8.47	0.38
ST RBF-10	0.00	0.00	8.12	0.55
ST RBF-40	0.00	0.00	8.20	0.43
ST RBF-80	0.00	0.00	8.39	0.36
Infested control	3.07	0.31	2.48	0.18
Infested RBF soil-mix	2.30	0.27	2.99	0.33
Infested ST RBF-10	1.70	0.34	3.49	0.31
Infested ST RBF-40	1.90	0.33	3.70	0.58
Infested ST RBF-80	1.15	0.34	4.28	0.42

Claims:

1. A method of controlling, limiting or preventing an infestation of a crop plant by a phytopathogenic fungi of the genus *Fusarium* by contacting plant propagation material, prior to or during sowing of the crop plant, with a composition comprising riboflavin.

2. The method according to claim 1, wherein the phytopathogenic fungi is *Fusarium oxysporum.*

3. The method according to claim 1 or claim 2, wherein the plant propagation material is a seed.

4. The method according to any one of claims 1 to 3, wherein the composition comprising riboflavin is applied as a seed treatment composition, further comprising an agrochemically acceptable diluent or carrier.

5. The method according to any one of claims 1 to 4, wherein the composition further comprises a fungicidal, insecticidal or nematicidal active ingredient at a weight ratio range to riboflavin of 1:50 to 50:1, and preferably 1:10 to 10:1.

6. The method according to claim 5, wherein the active ingredient is selected from:

acibenzolar-s-methyl, metalaxyl-M, metalaxyl, picarbutrazox, oxathiapiprolin, ethaboxam, fludioxonil, azoxystrobin, thiabendazole, difenoconazole, tebuconazole, ipconazole, mefentrifluconazole, prothioconazole, triticonazole, sedaxane, inpyrfluxam, penflufen, fluopyram, fluxapyroxad, pydiflumetofen, cyclobutrifluram, thiamethoxam, isoflucypram, clothianidin, *Bacillus amyloliquefaciens*, benzovindiflupyr, cymoxanil, phosphite (phosphonate), silthiofam, pyraclostrobin, isotianil, tolprocarb, quinofumelin, ipflufenoquin, metyltetraprole, florylpicoxamid, fenpicoxamid, fluoxytioconazole, fluindapyr, fluoxapiprolin, broflanilide, imidacloprid, acetamiprid, cyantraniliprole, chlorantraniliprole, tetraniliprole, fluxamethamide, penthiopyrad, mandipropamid, dimpropyridaz, flupyrimin, triflumezopyrim, oxazosulfyl, dichlobentiazox, benzpyrimoxan, hymexazol, pyrapropoyne, cyclaniliprole, mandestrobin, copper, lambda-cyhalothrin, phenamacril, abamectin, tefluthrin, picoxystrobin, spiropidion, oligosaccharins, *Melaleuca alternifolia*, fosthiazate, fenamifos, oxamyl, fluensulfone, fluazaindolizine, spirotetramat, spidoxamat, fluazinam, indazapyroxamet, thiophanate methyl, zoxamide, fluoxastrobin, pirimifos, tetrachlorantraniliprole, bifenthrin and emamectin benzoate.

7. The method according to any one of claims 3 to 6, wherein the weight ratio of riboflavin to seed is 10 mg to 5 g of riboflavin per kg of seeds, and preferably 100 mg to 1 g of riboflavin per kg of seeds.

8. The method according to any one of claims 1 to 7, wherein the crop plant is selected from cotton, maize (corn), wheat or soybean.

9. The method according to any one of claims 3 to 8, further comprising the step of sowing the seed in a soil substrate susceptible to, or with, the presence of phytopathogenic fungi of the genus Fusarium.

10. A seed treatment composition comprising riboflavin, and further comprising an agrochemically acceptable diluent or carrier.

11. The composition according to claim 10, wherein the composition further comprises a fungicidal, insecticidal or nematicidal active ingredient at a weight ratio range to riboflavin of 1:50 to 50:1, and preferably 1:10 to 10:1.

12. The composition according to claim 11, wherein the fungicidal, insecticidal or nematicidal active ingredient is selected from:

acibenzolar-s-methyl, metalaxyl-M, metalaxyl, picarbutrazox, oxathiapiprolin, ethaboxam, fludioxonil, azoxystrobin, thiabendazole, difenoconazole, tebuconazole, ipconazole, mefentrifluconazole, prothioconazole, triticonazole, sedaxane, inpyrfluxam, penflufen, fluopyram, fluxapyroxad, pydiflumetofen, cyclobutrifluram, thiamethoxam, isoflucypram, clothianidin, *Bacillus amyloliquefaciens*, benzovindiflupyr, cymoxanil, phosphite (phosphonate), silthiofam, pyraclostrobin, isotianil, tolprocarb, quinofumelin, ipflufenoquin, metyltetraprole, florylpicoxamid, fenpicoxamid, fluoxytioconazole, fluindapyr, fluoxapiprolin, broflanilide, imidacloprid, acetamiprid, cyantraniliprole, chlorantraniliprole, tetraniliprole, fluxamethamide, penthiopyrad, mandipropamid, dimpropyridaz, flupyrimin, triflumezopyrim, oxazosulfyl, dichlobentiazox, benzpyrimoxan, hymexazol, pyrapropoyne, cyclaniliprole, mandestrobin, copper, lambda-cyhalothrin, phenamacril, abamectin, tefluthrin, picoxystrobin, spiropidion, oligosaccharins, *Melaleuca alternifolia*, fosthiazate, fenamifos, oxamyl, fluensulfone, fluazaindolizine, spirotetramat, spidoxamat, fluazinam, indazapyroxamet, thiophanate methyl, zoxamide, fluoxastrobin, pirimifos, tetrachlorantraniliprole, bifenthrin and emamectin benzoate.

13. A plant seed treated with a composition according to any one of claims 10 to 12.

14. The seed according to claim 13, which is coated with a composition according to any one of claims 10 to 12.

15. Use of riboflavin as a seed treatment ingredient for the control of a phytopathogenic fungi of the genus Fusarium in a crop plant.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/065653

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01N43/90 A01P3/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01N A01P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SIKHAO P. ET AL: "Seed coating with riboflavin, a natural fluorescent compound, for authentication of cucumber seeds", SEED SCIENCE AND TECHNOLOGY : PROCEEDINGS OF THE INTERNATIONAL SEED TESTING ASSOCIATION , vol. 42, no. 2 1 August 2014 (2014-08-01), pages 171-179, XP093089401, Wageningen, NL ISSN: 0251-0952, DOI: 10.15258/sst.2014.42.2.06 Retrieved from the Internet: URL:http://dx.doi.org/10.15258/sst.2014.42.2.06	10,13,14
Y	page 172, line 22 - line 32 page 173, line 10 - line 18	1-15

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[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 July 2024	23/07/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Habermann, Jörg

1

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	page 10844, column 1, line 6 - line 9 table 1	1-15

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PUSHPALATHA ET AL: "Ability of vitamins to induce downy mildew disease resistance and growth promotion in pearl millet", CROP PROTECTION, ELSEVIER SCIENCE, GB, vol. 26, no. 11, 1 October 2007 (2007-10-01), pages 1674-1681, XP022282011, ISSN: 0261-2194, DOI: 10.1016/J.CROPRO.2007.02.012	10,13
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/065653

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 111 990 085 A (ZHEJIANG MAOYUAN FORESTRY ENG CO LTD) 27 November 2020 (2020-11-27) paragraph [0078] -----	10-12
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Y	paragraph [0009] -----	10-14
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Y	experimental example 4 -----	10-14

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/065653

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