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(54) Title: SEED TREATMENT WITH NATAMYCIN

(57) Abstract: The present invention relates to a method for protecting a product against oomycetes and to the use of natamycin in the protection of a product against oomycetes.

SEED TREATMENT WITH NATAMYCIN

Field of the invention

The present invention relates to a method for protecting a product against oomycetes and to the use of natamycin in the protection of a product against oomycetes.

Background of the invention

Fungi, yeast and bacteria can cause food and feed spoilage. Because of the large scale at which food & feed is produced, the consequence of spoilage is a severe economic loss for the food and feed industry. Microorganisms are the main agents responsible for food, feed spoilage and therefore food, and feed preservation procedures are targeted towards them. Food and feed preservation methods currently used by the industry rely either on the inhibition of microbial growth or on microbial inactivation. Examples of procedures for preservation of foods and feed are drying, salting, thermal treatment and fermentation.

Thermal treatment is the most widely used procedure. However, heat can trigger unwanted reactions, leading to undesirable organoleptic and nutritional effects. This limitation together with increasing consumer demand for fresh-like foods has promoted the development of alternative methods for food preservation, among which chemical preservation, an approach that finds extensive application.

Agriculture produces crops of many varieties such as *inter alia* legumes, fruits, lettuce, wheat, barley, corn and rice. Many of these crops are grown from seeds that vary in their innate ability to resist physical damage due to unfavorable storage or environmental conditions, all of which affects their subsequent ability to grow into adult plants. Furthermore, seeds are susceptible to damage by plant pathogens including fungi, bacteria, viruses, and nematodes and are vulnerable to insects, birds, rodents, and other organisms that rely on them as a food source. Fungi are one of the most economically important groups of plant pathogens and are responsible for huge annual losses of marketable food, fiber and feed.

An example of fungi that can spoil crop is the group of oomycetes. Oomycetes are fungus-like eukaryotic microorganisms that form the phylum Oomycota. Oomycetes are filamentous organisms and include some of the most notorious pathogens of plants, causing devastating diseases such as late blight of potato and sudden oak death. *Pythium* and *Phytophtera* are examples of oomycetes. Plant-pathogenic oomycetes include about sixty species of the genus *Phytophthora*, several genera of the biotrophic downy mildews, and more

than one hundred species of the genus *Pythium*. *Pythium* species complex is found in almost every soil and can cause disease throughout a plant's lifecycle. Seedling diseases caused by *Pythium* are a significant cause for concern as infection of seed prior to germination or soon thereafter leads to significant stand loss often resulting in yield losses, increased grower costs due to re-planting, increased greenhouse emissions due to re-planting, and loss of crop yield potential due to shortened cropping season. *Phytophthora* species cause some of the most destructive plant diseases in the world and are arguably the most devastating pathogens of dicotyledonous plants. *Phytophthora infestans* causes the serious potato disease known as late blight or potato blight, which was the major culprit in the 1845 Irish potato famine.

To reduce yield losses due to, for instance stand loss, today a significant fraction of seed is treated with one or more synthetic agrochemicals. Application of agrochemicals to seed is fraught with problems such as bonding of the agrochemicals to the soil, agglomeration of seed due to the application of the agrochemicals and the use of expensive and complex chemical application equipment. In addition, seeds can be adversely affected by agrochemicals, as these chemicals can be toxic to seed and to the plants that sprout from seed. Such toxicity limits the amount of these agrochemicals that can safely be applied to seed. One undesirable effect of the toxicity may be the reduction of the germination rate and/or speed, or even total lack of germination, of seeds that are treated. Typically, the germination rate and/or speed of seeds that have undergone treatment with an agrochemical that is toxic decreases with time after the application of the agrochemical, thereby limiting the shelf life of the treated seeds.

One way to overcome the toxic effect is to encapsulate the agrochemical in a matrix, which limits its movement. This method can limit the contact of the agrochemical with seed and the emerging seedlings, while permitting the chemical to become available later during germination and initial plant growth as the chemical releases from the matrix. The proper operation of encapsulation technology depends on careful matching of the physical and chemical properties of the agrochemical and the encapsulating matrix. Neither one matrix, nor one encapsulation process, is suitable for encapsulation of all agrochemicals now in use for seed treatment. Moreover, the encapsulation matrix is susceptible to cracking.

Yet another way to overcome the toxic effect is to cover seed with a relatively thick layer of inert material onto which the agrochemical is applied in such a way that it is not directly in contact with seed (see WO 2004/049778). However, a disadvantage of this method is that a high dosage of agrochemical is necessary to be effective, which still may lead to a possible toxic effect of the agrochemical for seed. Furthermore, due to the high dosage, the coating's physiochemical properties may change significantly, indirectly producing a negative effect due to a change in the oxygen/water balance in the coating.

A further alternative includes the simultaneous sowing of seed-containing pellets and agrochemical-containing pellets as separate pellets (see US 2006/0150489). A disadvantage of

this solution is that two separate pellets are required, which is cumbersome and expensive. Moreover, this solution can stimulate irregular use of the agrochemical-containing pellets as there is no or only limited control at the application time, rate, and location of the pellets.

EP 2659775 describes a composition comprising a polyene antifungal compound and at least one antifungal compound from the family of phenylamide fungicides. According to EP 2659775, phenylamide fungicides can be used against oomycetes.

WO 2013/092995 describes a method for improving seed germination, said method comprising the step of contacting the seed, the medium to be planted by the seed or both with natamycin. Natamycin is a polyene macrolide antimycotic that is used to prevent fungal growth on food products such as cheeses and sausages. This natural preservative, produced by fermentation using *Streptomyces natalensis*, is widely used throughout the world as a food preservative and has a long history of safe use in the food industry. It is very effective against all known food spoilage fungi. Although combinations of natamycin with fungicides such as phenylamide fungicides have been reported, for example in WO 97/47202, WO 2012/162412, WO 2014/085576, WO 2014/191449 and WO 2017/043972, various standard works and textbooks in the field have taught that natamycin is ineffective against oomycetes. For example, in WHO Food Additive Series 48, “Safety Evaluation of Certain Food Additives and Contaminants – Natamycin (Pimaricin)”, JEFCA Food Additive Series 48) it is disclosed that oomycete fungi and bacteria are insensitive to these antibiotics because their membranes lack sterols. Also, in “Phytophtera Diseases Worldwide” by Donald C. Erwin and Olaf K. Ribeiro, APS Press, St Paul, Minnesota, chapter 2, it is disclosed that natamycin is active against most fungi except the *Pythiacaea* in the oomycetes. In addition, in “Fungal sterols and the mode of action of the polyene antibiotics” by J.M.T Hamilton-Miller it is disclosed that oomycetes are resistant to the antifungal action of polyenes.

Thus, there is a strong and clear teaching from the art not to use natamycin to combat oomycetes. Despite this, the inventors have surprisingly found that natamycin can be used against oomycetes even in the absence of phenylamide fungicides such as metalaxyl.

Detailed description of the invention

Throughout the present specification and the accompanying claims, the words "comprise”, "include" and “having” and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.* to one or at least one) of the grammatical object of the article. By way of example, "a seed" may mean one seed or more than one seed.

The invention relates to a method for protecting a product against oomycetes, said method comprising contacting the product with natamycin substantially in the absence of metalaxyl. It was surprisingly found that natamycin can be used against oomycetes even in the absence of phenylamide fungicides such as metalaxyl.

In an embodiment, the oomycetes are selected from the group consisting of *Pythium* and *Phytophtera*.

In an embodiment, the product is a food product. The term "food product" as used herein is to be understood in a very broad sense and includes, but is not limited to, dairy products, meat products, fish products, beverage products, baking products, unpasteurized food products, salads, and sauces, marinades, salsas and seasonings. A “dairy product” is understood to include any food product made using milk or milk products, including, but not limited to, milk, yoghurt, ice cream, cheese, skimmed milk, acidified milk, butter milk, condensed milk, spreads, margarines, milk powder, butter, EMC (Enzyme Modified Cheese), Dulce de leche, coffee whitener; coffee creamer, cream, sour cream, ghee, and dairy analogue. Cheese may be any kind of cheese, e.g. fresh cheese, hard cheese, curd cheese, cream cheese, white mould cheese, blue mould cheese and process cheese. The term ‘analogue of a dairy product’ or 'dairy analogue' refers to a dairy-like product which contains a dairy composition as defined herein and which composition comprises at least one analogue of a dairy ingredient. In various embodiments, the milk is raw milk or milk that has been pasteurized. A “meat product” is understood to include any food product, which contains animal tissue, including, but not limited to, beef, pork, and poultry. The term “ready-to-eat meat product” is intended to include any meat product, which does not require cooking prior to consumption, including, but not limited to, pates, hot dogs, bologna, ham, salami, sausages, deli meats, and cold cuts. A “fish product” is included to include any food product, which contains tissue from an aquatic animal, including, but not limited to, lobster, crab, fresh water, smoked salmon, smoked other fish, salted fish, saltwater fish and other seafood. A “baking product” is understood to include any product prepared from a dough or a batter. The product may have a soft or a crisp character and may be of a white, light or dark type. Baked products include, but are not limited to, bread such as for instance white, whole-meal or rye bread, French baguette-type bread, laminated dough products such as (Danish) pastry, croissants or puff pastry, pita bread, tortillas, tacos, cakes, pancakes, biscuits, cookies, doughnuts, bagels, pie crusts, muffins, steamed bread, and crisp bread. Types of baked products, methods to characterize and to produce them are known to those skilled in the art see for example “Baking Science and Technology”, by E.J. Pyler, L.A. Gorton, 2008, (2 volumes) Sosland Publishing Company, Kansas, USA, or “Baked Products: Science, Technology and

Practice" by S.P. Cauvain, L.S. Young, 2006, Blackwell Publishing Ltd, Oxford, UK. "Unpasteurized food product" is included to include any food product, whereby at least one ingredient is unpasteurized and which does not undergo a final heat treatment. A "salad" is included to include any food product, which contains vegetables, fruits or mixtures thereof. Examples include, but are not limited to, products that are presented for consumers to choose from in a display commonly referred to as a "salad bar", deli salads, processed fruit and vegetables, cut salads and cut vegetables such as cut lettuce, cut romaine lettuce, cut spinach and cut endive. Of course, the salads can also be uncut.

Alternatively, in an embodiment the product is a feed product or an agricultural product such as a crop.

In another embodiment, the product is seed. The invention includes protecting a seed against oomycetes, said method comprising contacting the seed, medium to be planted by the seed, or both with natamycin. In the context of the invention "protecting a product against oomycetes" includes less infection with oomycetes, increased germination rate of seed, reduced sudden death of plants, higher crop yield from seed, or a combination thereof, all as compared to no contacting with natamycin.

Natamycin may improve seed germination by 1 to 15%, preferably 5 to 15% after 14 to 16 days of incubation of seeds at 0 to 40ºC, preferably 5 to 30ºC, more preferably 10 to 20ºC. In an embodiment natamycin improves seed germination by at least 1%, at least 3%, at least 5%, at least 8%, at least 10%, preferably at least 15%, more preferably at least 20% and most preferably at least 25% after 14 to 16 days of incubation of seeds at 0 to 40ºC, preferably 5 to 30ºC, more preferably 10 to 20ºC. Seeds can be incubated in a professional sterile germination room with set temperatures and light conditions. Seeds can be planted in a sterile roll paper according to well-known ISTA (International Seed Testing Association) procedures (see Handbook International Rules for Seed Testing, Edition 2011, Chapter 5, published by the International Seed Testing Association, Switzerland). The planted seeds can be subjected to the following cycle for 14 to 16 days: 12 hours dark at 20°C followed by 12 hours of light at 30°C; humidity was between 98 and 100%.

In the context of the invention, "natamycin" includes derivatives thereof, such as, but not limited to, salts or solvates of natamycin, or modified forms of natamycin. Examples of commercial products containing natamycin are the products with the brand name Delvocid®. Such products are produced by DSM Food Specialties (The Netherlands) and may be solids containing *e.g.* 50% (w/w) natamycin or liquids comprising between *e.g.* 0.5-50% (w/v) natamycin. Said commercial products can be contacted with the seed, medium to be planted by the seed or both.

"A medium to be planted by the seed" refers to any growing environment suitable for growing a plant and/or seedling from a seed such as soil and other growth media (natural or artificial). Natamycin can be applied to soil in-furrow, growing blocks, and gutters or in T-bands.

Natamycin can be applied at the same time as seed is sown. In an embodiment, natamycin can be applied to seed, medium to be planted by the seed or both through irrigation water.

“Seed” as used herein means any resting stage of a plant that is physically detached from the vegetative stage of a plant. The term "resting" refers to a state wherein the plant retains viability, within reasonable limits, in spite of the absence of light, water and/or nutrients essential for the vegetative (*i.e.* non-seed) state. Seeds may be stored for prolonged periods and can be used to re-grow another plant individual of the same species. In particular, the term “seed” refers to true seeds, but does not embraces plant propagules such as suckers, corms, bulbs, fruit, tubers, grains, cuttings and cut shoots. In other words, seeds are a ripened ovule of gymnosperms and angiosperm, which develops following fertilization and contains an embryo surrounded by a protective cover. Alternatively, artificial seeds do not need fertilization. Other food reserve storing tissues such as *e.g.* endosperm may be present in mature seeds.

Seeds of plant varieties of all types, which are used in agriculture, in greenhouses, in forestry, in garden construction or in vineyards, can be contacted with natamycin. In particular, this concerns seeds of corn, maize, triticale, teff, peanut, canola, rape, poppy, olive, coconut, grasses, cacao, soy, cotton, beet, (*e.g.* sugar beet and fodder beet), rice (any rice may be used, but is preferably selected from the group consisting of *Oryza sativa* sp. japonica, *Oryza sativa* sp. javanica, *Oryza sativa* sp. indica, and hybrids thereof), sorghum, millet, teff, spelt, wheat, durum wheat, barley, oats, rye, sunflower, sugar cane, turf, pasture, alfalfa or tobacco. Natamycin can also be used for the treatment of seed of fruit plants including, but not limited to, rosaceous fruit, for example apples and pears; stone-fruits, for example peaches, nectarines, cherries, plums and apricots; citrus fruit, for example, oranges, grapefruit, limes, lemons, kumquats, mandarins and satsumas; nuts, for example pistachios, almonds, walnuts, coffee, cacao and pecan nuts; tropical fruits, for example, mango, papaya, pineapple, dates and bananas; and grapes; and vegetables including, but not limited to, leafy vegetables, for example endives, lambs lettuce, rucola, fennel, globe (head lettuce) and loose-leaf salad, chard, spinach and chicory; brassicas, for example, cauliflower, broccoli, Chinese cabbage, kale (winter kale or curly kale), kohlrabi, Brussels sprouts, red cabbage, white cabbage and savoy; fruiting vegetables, for example, egg plants (aubergines), cucumbers, paprika, peppers (hot), marrow, tomatoes, courgettes, melons, watermelons, pumpkins and sweet corn; root vegetables, for example celeriac, turnip, carrots, swedes, radishes, horse radish, beetroot, salsify, celery; pulses, for example, peas and beans; and bulb vegetables, for example leeks, garlic and onions. Natamycin can also be used for the treatment of seed of ornamental seeds, for example, roses, pansy, impatiens, petunia, begonia, Lisianthus, sunflower, ageratum, chrysanthemum and geranium. In a preferred embodiment, natamycin is used for the treatment of seeds of tomatoes, cabbages (*e.g.* Chinese cabbage, kale (winter kale or curly kale), kohlrabi, Brussels sprouts, red cabbage, white cabbage and savoy), onions, paprika, aubergines (also called eggplants), lettuce (*e.g.* endives, lambs lettuce, rucola,

fennel, globe (head lettuce) and loose-leaf salad, chard, spinach and chicory), corn, rice, soy and cucurbitaceae (*e.g.* cucumbers, pumpkins, watermelons and melons). The method of the invention is particularly useful for corn seed or soy seed.

Seed may be transgenic seeds, *i.e.* seeds of a transgenic plant. As used herein "transgenic plant" means a plant or progeny thereof derived from a transformed plant cell or protoplast, wherein the plant DNA contains an introduced exogenous DNA molecule not originally present in a native, non-transgenic plant of the same strain.

Although the present method can be applied to seeds in any physiological state, it is preferred that seed be in a sufficiently durable state that they incur no significant damage during the seed treatment process. Seed may have been harvested from the field; removed from the plant; and/or separated from the fruit and any cob, pod, stalk, outer husk, and surrounding pulp or other non-seed plant material. Seed is preferably also biologically stable to the extent that the treatment would cause no biological damage to seed. In one embodiment, for example, the treatment can be applied to seeds that have been harvested, cleaned and dried to a specific moisture content. In an alternative embodiment, seed can be dried and then primed with water and/or another material and then re-dried before, during or after treatment with natamycin.

Natamycin can be applied "neat", that is, without any diluting or additional components present. However, natamycin is typically applied to a product such as seed in the form of a composition and/or coating and/or formulation. Such a composition and/or coating and/or formulation may be a (physical) mixture of natamycin and at least one other component. However, such composition and/or coating and/or formulation may also be any combination of natamycin and at least one other component, it not being required for natamycin and the at least one other component to be present together in the same composition and/or coating and/or formulation. An example of a composition and/or coating and/or formulation in which natamycin and at least one other component are not present together in the same composition and/or coating and/or formulation is a kit of parts. In a kit of parts, two or more components of a kit are packaged separately, *i.e.* not pre-formulated. As such, kits include one or more separate containers such as vials, cans, bottles, pouches, bags or canisters, each container containing a separate component for an agrochemical composition. Examples are two-component, three-component or even four-component kits.

In an embodiment, seed is contacted with natamycin by spraying or dipping. Seed can also be contacted with natamycin by coating (e.g. seed pelleting, encrusting, film coating), seed dusting and seed imbibition (e.g. seed soaking, priming). "Seed treatment" refers to all methods that bring seed and natamycin into contact with each other, and "seed dressing" refers to methods of seed treatment which provide seed with an amount of natamycin, i.e. which generate a seed comprising natamycin. In principle, the treatment can be applied to seed at any time from the harvest of seed to the sowing of the seed. Seed can be treated immediately before, or

during, the planting of seed. However, the treatment may also be carried out several weeks or months, for example from 1 day to 48 months, or from 1 week to 36 months or from 1 month to 24 months before planting seed, for example in the form of a seed dressing treatment. Corn, for example, is often stored for 24 months following seed treatment prior to planting. The treatment can be applied to unsown seeds. As used herein, the term "unsown seeds" is meant to include seed at any period from the harvest of the seed to the sowing of the seed in the ground for the purpose of germination and growth of a plant. Seed can also be treated after sowing by e.g. applying natamycin to soil or medium, rather than directly to seed. By applying seed-treatment prior to the sowing of seed, the operation is simplified. In this manner, seed can be treated, for example, at a central location and then dispersed for planting. This permits the person planting the seed to avoid the handling and use of natamycin and to merely handle and plant the treated seed in a manner that is conventional for regular untreated seed, which reduces human exposure. Seed to be treated can be primed or unprimed seed. Priming of seed is done to bring seed to the same germination level under controlled conditions. Examples of priming techniques are osmopriming and drum priming. These priming techniques are known to the skilled artisan.

A suitable device for seed treatment, for example a mixer for solid or solid/liquid components, can be employed until natamycin is distributed uniformly onto seed. Natamycin can be applied to seed by any standard seed treatment methodology, including, but not limited to, mixing in a container (*e.g.* bottle, bag, tumbler, rotary coater, fluidized bed or sprayer), mechanical application, tumbling, spraying, and immersion. This can be followed by drying of seed. Spray seed treatment is a method usually used for treating large volume of rice seeds. For this purpose, a solution obtained by dilution of a composition (*e.g.* a FS, LS, DS, WS, SS and ES) is sprayed continuously on seed in a spray chamber and then dried at elevated temperature (*e.g.* 25 to 40°C) in a drying chamber.

Seed can be subjected to coating or imbibition (*e.g.* soaking). "Coating" denotes any process that endows the outer surfaces of seed partially or completely with a layer or layers of non-plant material. Coating is most commonly used for broad acre crops like rice, corn and vegetable seeds. According to this method, seed can be cleaned and afterwards coated with a diluted formulation by using *e.g.* a rotating pot-mixer for about several minutes and followed by reversible rotation. Afterwards, seed can be dried.

"Imbibition" refers to any process that results in penetration of natamycin into the germinable parts of the seed and/or its natural sheath, (inner) husk, hull, shell, pod and/or integument. According to the soaking method, seed is cleaned and packed in a bag that is sunk into the equivalent volume of chemical solution with seed volume, wherein the chemical solution normally is obtained by the dilution of a formulation such as FS, LS, DS, WS, SS and ES. Afterwards, the seed can be dried. Soaking is most commonly applied for rice seed.

Coating with natamycin may be applied to seed using conventional coating techniques and machines, such as fluidized bed techniques, the roller mill method, rotostatic seed treaters, and drum coaters. Other methods such as the spouted beds technique may also be useful. Seed may be pre-sized before coating. After coating, seed can be dried and transferred to a sizing machine for sizing. Drying can be carried out by natural ventilation, or in accordance with any technique, which is in itself known, such as passing an optionally heated, forced stream of air over seed, which can be arranged, for this purpose, in apparatuses such as sieves.

When coating of seed is to be done on large scale (for example a commercial scale), seed may be introduced into treatment equipment (such as a tumbler, a drum, a plate, a mixer or a pan granulator) either by weight or by flow rate. The amount of natamycin that is introduced into treatment equipment can vary depending on the seed weight, surface area, the concentration of natamycin, the desired concentration on the finished seed, and the like. Natamycin can be applied to seed by a variety of means, for example by a spray nozzle or revolving disc. The amount of natamycin is typically determined by the required rate of natamycin necessary for efficacy. As seed falls into the treatment equipment it can be treated (for example by misting or spraying with natamycin) and passed through treatment equipment under continual movement/tumbling where it can be coated evenly and dried before storage or use. In another embodiment, a known weight of seed is introduced into treatment equipment. A known volume of natamycin can be introduced into treatment equipment at a rate that allows natamycin to be applied evenly to seed. Powder for encrusting can be added manually or through an automated powder feeder. During the application, seed can be mixed, for example by spinning or tumbling. Seed can optionally be dried or partially dried during the tumbling operation. After complete coating or encrusting, treated seed can be removed to an area for further drying or additional processing, use, or storage. In another embodiment, seed can be coated in laboratory size commercial treatment equipment such as a tumbler, a mixer, or a pan granulator by introducing a known weight of seed in treatment equipment, adding a desired amount of natamycin, tumbling or spinning seed and placing it on a tray to thoroughly dry. In another embodiment, seed can also be coated by placing a known amount of seed into a narrow neck bottle or receptacle with a lid. While tumbling, a desired amount of natamycin can be added to the receptacle. Seed can be tumbled until it is coated, encrusted or pelleted with natamycin. After coating, encrusting or pelleting, seed can optionally be dried, for example on a tray. If necessary, drying can be done by conventional methods. For example, a desiccant or mild heat (such as below about 40°C) may be employed to produce a dry coating or encrusting. Alternatively, coating may also be done by applying a "sticking agent" such as a filler or binder as an adhesive film over seed so that natamycin in the form of a powder can be bonded to seed to form a coating, encrusting or pellet. For example, a quantity of seed can be mixed with a sticking agent, and optionally agitated to encourage uniform coating of seed with the sticking agent. In the second step, seed coated with the sticking agent can be mixed with the powdered

mixture of natamycin. A dry formulation of natamycin may contain other components as discussed below. A mixture of seed and natamycin can be agitated, for example by tumbling, to encourage contact of the sticking agent with the powdered material, thereby causing the powdered material to stick to the seed.

Natamycin may be comprised in a composition and/or coating and/or formulation (all denoted as composition hereafter). A composition comprising natamycin may comprise one or more further components. Such components include, but are not limited to, other pesticides (such as fungicides, acaricides, miticides, insecticides, insect repellents, bird repellents, rodenticides, molluscicides, nematicides, bactericides, and fumigants), herbicides, adjuvants, wetters, nutrients, waxes, anti-oxidation agents, gene activators protective colloids, surfactants, minerals, chemical hybridizing agents, pigments, auxins, sticking agents, antibiotics and other drugs, biological attractants, colorants, dispersing agents, solvents, solid carriers, growth regulators, pheromones, thickening agents, dyes, safeners, fertilizers, anti-freeze agents, biocontrol agents (*e.g.* naturally-occurring or recombinant bacteria and/or fungi), liquid diluents, binders (*e.g.* to serve as a matrix for natamycin), fillers (*e.g.* fine powders of organic or mineral type for protecting seed during stress conditions), plasticizers (to improve flexibility, adhesion, and/or spreadability), drying agents, solubilizers, dispersing agents, anti-foaming agents.

In an embodiment, the method of the invention comprises contacting seed with a composition comprising natamycin and substantially no antifungal compound from the family of phenylamide fungicides, more preferably a phenylamide fungicide selected from the group consisting of benalaxyl, benalaxyl-M (also called kiralaxyl), furalaxyl, metalaxyl, and metalaxyl-M (also called mefenoxam), ofurace and oxadixyl. In the context of the present invention, “substantially no antifungal compound from the family of phenylamide fungicides” is less than 10 ppm antifungal compound from the family of phenylamide fungicides, or from 0 to 10 ppm antifungal compound from the family of phenylamide fungicides or from 1 to 5 ppm antifungal compound from the family of phenylamide fungicides.

Examples of commercial products containing phenylamide fungicides such as benalaxyl are products with the brand names Galben®, Tairel® and Trecatol®. Examples of commercial products containing phenylamide fungicides such as metalaxyl are products with the brand names Allegiance® FL, Apron® XL, Ridomil® and Subdue®. Examples of commercial products containing phenylamide fungicides such as oxadixyl are products with the brand names Sandofan®, Recoil®, Ripost® and Wakil®. Said commercial products can be incorporated in the present invention.

A suitable composition for contacting a product such as seed according to the invention can be in the form of a soluble concentrate (SL, LS), a dispersible concentrate (DC), an emulsifiable concentrate (EC), a suspension (SC, OD, FS), an emulsion (EW, EO, ES), a slurry of particles in an aqueous medium (*e.g.* water), a paste, a water-dispersible or water-soluble

powder (WP, SP, SS, WS), a pastille, a water-dispersible or water-soluble granule (WG, SG), a dry granule (GR, FG, GG, MG), a gel formulation (GF), a dustable powder (DP, DS), to name just a few. Water-soluble concentrates (LS), flowable concentrates (FS), powders for dry treatment (DS), water-dispersible powders for slurry treatment (WS), water-soluble powders (SS), emulsions (ES), emulsifiable concentrates (EC) and gels (GF) are usually employed for the purposes of treatment of seed. These compositions can be applied to seed, diluted or undiluted.

In an embodiment, the method of the invention comprises contacting seed with a composition comprising natamycin and further comprising a filler, a binder or both. Alternatively, seed can be contacted with separate components. Contacting can be done at the same time or separately. For example, seed can be contacted with a composition comprising a filler and natamycin and subsequently with a composition comprising a filler and a binder. Alternative combinations are within the reach of the skilled artisan.

In an embodiment, a filler is selected from the group consisting of a carbonate, wood flour, diatomaceous earth and a combination thereof. In a preferred embodiment, the filler is a carbonate. Examples of carbonates are calcium carbonate, magnesium carbonate or a combination thereof. In an embodiment, the filler is calcium carbonate.

In an embodiment, a binder is selected from the group consisting of lignosulphonate, polyvinylpyrrolidone and a combination thereof. Polyvinylpyrrolidone is a water-soluble polymer made from the monomer N-vinylpyrrolidone ($(C_6H_9NO)_N$). The molecular weight depends on the number of monomer units. Lignosulphonate can be added as copper lignosulphonate, zinc lignosulphonate, magnesium lignosulphonate, manganese lignosulphonate, sodium lignosulphonate, calcium lignosulphonate, ammonium lignosulphonate, or a combination thereof.

In general, a product such as seed is contacted with about 1 to 400 ppm natamycin based on the total weight of the seed, that is: about 10 to 4000 grams of natamycin per 100 kg of product (such as seed). Preferably, a product is contacted with 10–250 ppm, more preferably with 25-100 ppm natamycin.

The invention further provides the use of natamycin to protect a product against oomycetes.

Embodiments and features described herein pertain to the all embodiments of the invention.

EXAMPLES

General

Paper towel test: Germination paper towels were soaked in water until completely wet. Excess water was removed by squeezing the towels or by allowing to drain on a slanted board for about 5-10 minutes (paper should appear glossy/wet, but not too wet that a film of water forms around the finger when pressing the paper). A trip board was used to plant 100 seeds/sample. Four replications per sample were prepared. The assembly was covered with another paper towel and carefully rolled from one side going to the other. The rolled towels were placed in a plastic bag, sealed with a rubber band and placed in a room with temperature set at 25ºC. Germination was evaluated after 3 days by counting the normal seedlings. Normal seedings were removed, the towel was moisted again using a sprayer, and put back in 25ºC for another 4 days. The final seedling germination was counted, and any dead, hard, or abnormal seeds/seedlings were recorded. Refer for AOSA Rules Vol. 4 for guidelines in seedling evaluation.

Sand test: A Kimpak was placed on a tray and wetted with approximately 900 ml of water. The Kimpak was allowed to absorb the water for at least an hour. A trip board was used to plant 100 seeds/sample. Four replications per sample were prepared. The seeds were covered with sand and placed in a 25ºC room temperature with 12 h photoperiod. Germination was counted at 7 days after planting.

Sand-soil test: A Kimpak was placed on a tray, wetted with approximately 900 ml distilled water and left overnight at 10ºC prior to use. This test was run under cool conditions (10ºC for 7 days and then 18ºC for 10 days) since the cool and wet conditions strongly favor *Pythium* over other soil pathogens (also, *Pythium* mycelia were noted on the surface of the untreated check). A trip board was used to plant 100 seeds/sample. Four replications per sample were prepared. The seeds were covered by filling the tray with sand-soil mixture (1:1 ratio) and the tray was kept at 10°C without light for 7 days, and then transferred to 18°C for 10 days. Emergence of germination was counted at 3, 7, 10, and 14 days after transferring to 18°C.

Example 1

Germination of 1-year old soybeans (2015) treated with natamycin and/or metalaxyl

One year old soybean seeds were treated with natamycin and/or metalaxyl and investigated according to the paper towel test (not the sample with only natamycin), the sand test (not the sample with only natamycin) and the sand-soil test. For results, see Table 1.

Table 1

#	Natamycin ($g.100kg^{-1}$)	Metalaxyl ($g.100kg^{-1}$)	Germinated seeds (%)					
			Paper Towel	Sand	Sand-soil			
			7 days	7 days	4 days	7 days	10 days	14 days
1	0	0	62.5	71.25	0.251	0.063	0.2	0.2
2	0	15	56	58.25	10.51	24.883	28.136	28.152
3	5	15	68	70.25	14.819	35.682	40.892	40.889
4	10	15	58	75.5	23.483	46.235	50.642	51.376
5	25	15	51.5	72.25	22.547	44.956	53.332	53.592
6	50	15	72	73.5	21.786	42.233	47.961	49.185
7	50	0	N.A.	N.A.	0.366	2.891	3.175	3.624

Example 2

Germination of fresh soybeans (2016) treated with natamycin and/or metalaxyl

Fresh soybean seeds were treated with natamycin and/or metalaxyl and investigated according to the paper towel test (not the sample with only natamycin), the sand test (not the sample with only natamycin) and the sand-soil test. For results, see Table 2.

Table 2

#	Natamycin ($g.100kg^{-1}$)	Metalaxyl ($g.100kg^{-1}$)	Germinated seeds (%)					
			Paper Towel	Sand	Sand-soil			
			7 days	7 days	4 days	7 days	10 days	14 days
1	0	0	98	96.25	18.5	37.25	42.669	42.152
2	0	15	99	96.5	80	92.75	95.123	93.529
3	5	15	98.5	97	77.25	94.5	96.063	95.251
4	10	15	97.5	93.75	79	92.75	96.086	93.606
5	25	15	97	93.25	76.75	91.75	94.258	93.009
6	50	15	97.5	93.75	67.25	90.25	91.407	90.386
7	50	0	N.A.	N.A.	65.25	84.25	88.068	85.046

None of natamycin at tested rates caused any issues in the germination of 2015 or 2016 soybean seed based on results of paper towel test, sand test and cold test. *Pythium* significantly reduced

the germination of 2015 and 2016 untreated soybean seed in the sand-soil test; However, natamycin at 50 g.100kg^{-1} significantly improved the germination as compared to untreated seed, especially for 2016 seed, which suggests natamycin has activity against *Pythium* species. Natamycin at all tested rates significantly improved the germination of 2015 soybean seed as compared to seed treated with metalaxyl in the sand-soil test; No significant difference was observed for 2016 seed.

Example 3

Germination of 1-year old corn (2015) treated with natamycin and/or metalaxyl

One year old corn seeds were treated with natamycin and/or metalaxyl and investigated according to the paper towel test (not the sample with only natamycin), the sand test (not the sample with only natamycin) and the sand-soil test. For results, see Table 3.

Table 3

#	Natamycin (g.100kg^{-1})	Metalaxyl (g.100kg^{-1})	Germinated seeds (%)					
			Paper Towel	Sand	Sand-soil			
			7 days	7 days	4 days	7 days	10 days	14 days
1	0	0	98.5	98.8	3.7	5.2	5.2	5.4
2	0	15	98.5	96.8	89.4	93.7	93.7	94.2
3	5	15	99	96.3	91.7	94.1	94.1	94.9
4	10	15	98	95.5	89.8	92.2	92.2	92.6
5	25	15	98.5	97.5	93.1	93.7	94.1	94.1
6	50	15	98	98.3	93.7	96.7	96.7	97.1
7	50	0	N.A.	N.A.	44.9	47.4	47.4	48.7

Example 4

Germination of fresh corn (2016) treated with natamycin and/or metalaxyl

Fresh corn seeds were treated with natamycin and/or metalaxyl and investigated according to the paper towel test (not the sample with only natamycin), the sand test (not the sample with only natamycin) and the sand-soil test. For results, see Table 4.

Table 4

#	Natamycin (g.100kg^{-1})	Metalaxyl (g.100kg^{-1})	Germinated seeds (%)					
			Paper Towel	Sand	Sand-soil			
			7 days	7 days	4 days	7 days	10 days	14 days
1	0	0	98.2	98	36.1	41.1	41.1	41.1
2	0	15	99.9	98.3	91.5	95.8	96.1	96.1
3	5	15	99.5	99	88.8	97.2	97.2	97.2
4	10	15	99.5	98.5	88.8	98.3	98.3	98.3
5	25	15	99.3	98.3	91.8	98.3	98.3	98.3
6	50	15	99.6	98.8	87.5	97.7	98	98
7	50	0	N.A.	N.A.	85.1	94.1	94.6	94.6

None of natamycin at tested rates caused any issues in the germination of 2015 or 2016 corn seed based on results of paper towel test, sand test and cold test either. There is no significant difference in the corn seed germination observed among 4 rates of Natamycin. *Pythium* significantly reduced the germination of 2015 and 2016 untreated corn seed in the sand-soil test; However, natamycin at 50 g.100kg^{-1} significantly improved seed germination as compared to untreated seed, especially for 2015 seed, which suggests natamycin has activity against *Pythium* species.

CLAIMS

1. A method for protecting a product against oomycetes, said method comprising contacting the product with natamycin substantially in the absence of metalaxyl.

2. Method according to claim 1 wherein said method is carried out substantially in the absence of a phenylamide fungicide.

3. Method according to any one of claims 1 to 2 wherein said oomycetes are selected from the group consisting of *Pythium* and *Phytophtera*.

4. Method according to any one of claims 1 to 3 wherein said product is a food product, feed product or a seed.

5. Method according to claim 4 wherein said seed is corn seed or soy seed.

6. Method according to any one of claims 1 to 5 wherein said product is contacted with 10-250 ppm of natamycin.

7. Method according to any one of claims 1 to 6 wherein said natamycin is comprised in a composition, which composition further comprises an antifungal compound.

8. Method according to any one of claims 1 to 7 wherein said product is contacted with said natamycin by spraying or dipping.

9. Method according to any one of claims 1 to 8 wherein said natamycin is applied on the product by coating.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/066865

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01P3/00 A01N43/90 A01N43/08 A01N43/76 A01N37/46
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

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 Y	EP 2 659 775 A1 (DSM IP ASSETS BV [NL]) 6 November 2013 (2013-11-06) cited in the application the whole document -----	1-9 1-9
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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

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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
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/066865

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	paragraphs [0012,29,53,56,57,89]; claims 16, 33, 52	1-9

X	WO 2014/191449 A1 (DSM IP ASSETS BV [NL]) 4 December 2014 (2014-12-04)	1-4
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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