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(54) **NEW ANTIFUNGAL COMPOSITIONS**

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(57) **ABSTRACT**

The present invention relates to new antifungal compositions and their use in the treatment of agricultural products.

NEW ANTIFUNGAL COMPOSITIONS

FIELD OF THE INVENTION

[0001] The present invention discloses new antimicrobial compositions to control plant diseases and to prevent microbial spoilage of crops.

BACKGROUND OF THE INVENTION

[0002] It is estimated that about 25% of the world crop production is lost due to microbial spoilage, of which spoilage by fungi is by far the most important cause. Not only from an economical point of view, but also from a humane point of view it is of great importance to prevent spoilage of food products. After all, in many parts of the world people suffer from hunger.

[0003] Success in combating plant and crop diseases and in reducing the damage they cause to yields and quality depends greatly on the timely application of fungicides. The prolonged and frequent use of many fungicides such as e.g. benzamidazoles has contributed to reduce their effectiveness thanks to the development of phenomena of resistance.

[0004] Respiratory inhibitors are among the fungicides most widely used for disease control on crops. Most are strobilurins and carboxamides, inhibiting the cytochrome b of mitochondrial complex III and the succinate dehydrogenase of mitochondrial complex II, respectively.

[0005] The first generation of carboxamide fungicides, including carboxin, was discovered in the mid-1960s, and these molecules were effective only against basidiomycetes. New carboxamides with a much wider spectrum of activity have recently been discovered. Despite the constant development of new carboxamides, these fungicides have not been immune to challenges in their development and maintenance. A large concern has been resistance development. Resistance to carboxamide fungicides has been observed on several crops and diseases now (see Avenot et al., 2007; Fillinger et al., 2008; Leroux et al., 1988).

[0006] For many decades, the polyene macrolide antimycotic natamycin has been used to prevent fungal growth on food products such as cheeses and sausages. This natural preservative, which is 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 natamycin has been applied for many years in e.g. the cheese industry, up to now development of resistant fungal species has never been observed.

[0007] Consequently, it can be concluded that there is a severe need for more effective antimicrobial compositions, e.g. antifungal compositions, for the treatment of fungal growth in and on plants and crops.

DESCRIPTION OF THE INVENTION

[0008] The present invention solves the problem by providing a new synergistic antimicrobial, e.g. antifungal, composition comprising a polyene antifungal compound and at least one antifungal compound from the family of carboxamide fungicides. As used herein, the term "synergistic" means that the combined effect of the antifungal compounds when used in combination is greater than their additive effects when used individually.

[0009] In general, synergistic activity of two active ingredients can be tested in for example the analysis of variance model using the treatment interaction stratum (see Slinker, 1998). Relative efficacy can be calculated by means of the following formula: ((value of evolution status of untreated control−value of evolution status of composition)/(value of evolution status of untreated control))*100. An interaction coefficient can then be calculated by means of the following formula: ((relative efficacy of combination compound A+compound B)/(relative efficacy of compound A+relative efficacy of compound B))*100. An interaction coefficient larger than 100 indicates synergy between the compounds.

[0010] Alternatively, synergy can be calculated as follows: the antifungal activity (in %) of the individual active ingredients can be determined by calculating the reduction in mould growth observed on products treated with the active ingredients in comparison to the mould growth on products treated with a control composition. The expected antifungal activity (E in %) of the combined antifungal composition comprising both active ingredients can be calculated according to the Colby equation (Colby, 1967): $E=X+Y-[(X\cdot Y)/100]$, wherein X and Y are the observed antifungal activities (in %) of the individual active ingredients X and Y, respectively. If the observed antifungal activity (O in %) of the combination exceeds the expected antifungal activity (E in %) of the combination and the synergy factor O/E is thus >1.0, the combined application of the active ingredients leads to a synergistic antifungal effect.

[0011] The term "carboxamide fungicide" as used herein includes furan-carboxamide fungicides, oxathiin-carboxamide fungicides, thiazole-carboxamide fungicides, and pyridine-carboxamide fungicides. The term specifically excludes pyrazole-carboxamides. Examples of pyrazole-carboxamides are bixafen, fluxapyroxad, furametpyr, isopyrazam, penflufen, penthiopyrad and sedaxane.

[0012] In an embodiment of the invention, the at least one antifungal compound from the family of carboxamide fungicides is selected from the group consisting of boscalid, carboxin, fenfuram, fenhexamid, furcarbanil, isotianil, methfuroxam, metsulfovax, oxycarboxin, pyracarbolid, thifluzamide and tiadinil.

[0013] In an embodiment the compositions may also contain two or more different antifungal compounds from the family of carboxamide fungicides. It is to be understood that derivatives of antifungal compounds from the family of carboxamide fungicides including, but not limited to, salts or solvates of antifungal compounds from the family of carboxamide fungicides or modified forms of antifungal compounds from the family of carboxamide fungicides may also be applied in the compositions of the invention. Examples of commercial products containing carboxamide fungicides such as boscalid are products with the brand names Cantus®, Endura® and Emerald®. Examples of commercial products containing carboxamide fungicides such as carboxin are products with the brand names Cadan®, Sanvex® and Thiobel®. Examples of commercial products containing carboxamide fungicides such as fenhexamid are products with the brand names Teldor®, Elevate® and Password®. Said commercial products can be incorporated in the present invention.

[0014] In an embodiment the polyene antifungal compound is selected from the group consisting of natamycin, nystatin, amphotericin B, trienin, etruscomycin, filipin, chainin, dermostatin, lymphosarcin, candicidin, aureofungin A, aureofungin B, hamycin A, hamycin B and lucensomycin. In a preferred embodiment the polyene antifungal compound is natamycin. In an embodiment the compositions may also contain two or more different polyene antifungal compounds. It is to be understood that derivatives of polyene antifungal compounds including, but not limited to, salts or solvates of polyene antifungal compounds or modified forms of polyene antifungal compounds may also be applied in the compositions of the invention. 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. 2-50% (w/v) natamycin. Said commercial products can be incorporated in the compositions of the invention.

[0015] The composition of the present invention generally comprises from about 0.005 g/l to about 100 g/l and preferably from about 0.01 g/l to about 50 g/l of a polyene antifungal compound. Preferably, the amount is from 0.01 g/l to 3 g/l.

[0016] The composition of the present invention generally comprises from about 0.0001 g/l to about 2000 g/l and preferably from about 0.0005 g/l to about 1500 g/l of an antifungal compound from the family of carboxamide fungicides. More preferably, the amount is from 0.001 g/l to 1000 g/l.

[0017] In an embodiment the composition of the present invention further comprises at least one additional compound selected from the group consisting of a sticking agent, a carrier, a colouring agent, a protective colloid, an adhesive, a herbicide, a fertilizer, a thickening agent, a sequestering agent, a thixotropic agent, a surfactant, a further antimicrobial compound, a detergent, a preservative, a spreading agent, a filler, a spray oil, a flow additive, a mineral substance, a solvent, a dispersant, an emulsifier, a wetting agent, a stabiliser, an antifoaming agent, a buffering agent, an UV-absorber and an antioxidant. A further antimicrobial antifungal compound may be an antifungal compound (e.g. imazalil, thiabendazole) or a compound to combat insects, nematodes, mites and/or bacteria. Of course, the compositions according to the invention may also comprise two or more of any of the above additional compounds. Any of the above mentioned additional compounds may also be combined with the polyene antifungal compound and/or the at least one antifungal compound from the family of carboxamide fungicides in case the antifungal compounds are applied separately. In an embodiment the additional compounds are additives acceptable for the specific use, e.g. food, feed, medicine, cosmetics or agriculture. Additional compounds suitable for use in food, feed, medicine, cosmetics or agriculture are known to the person skilled in the art.

[0018] In a specific embodiment the further antimicrobial compound is a natural crop protection compound belonging to the group of phosphites, e.g. KH_2PO_3 or K_2HPO_3 or a mixture of both phosphite salts. Phosphite containing compounds as used herein means compounds comprising a phosphite group, i.e. PO_3 (in the form of e.g. $H_2PO_3^-$, HPO_3^{2-} or PO_3^{3-}) or any compound which allows the release of a phosphite ion including compounds such as phosphorous acid and phosphonic acid as well as derivatives thereof such as esters and/or alkali metal or alkaline earth metal salts thereof. In case the compositions of the present invention comprise a polyene antifungal compound (e.g. natamycin) and at least one phosphite containing compound, they preferably comprise 0.1 g or less lignosulphonate, more preferably 0.1 g or less polyphenol, per gram polyene antifungal compound. Preferably, they comprise 0.01 g or less lignosulphonate, more preferably 0.01 g or less polyphenol, per gram polyene antifungal compound. In particular, they are free of lignosulphonate and preferably free of polyphenol. Suitable examples of phosphite containing compounds are phosphorous acid and its (alkali metal or alkaline earth metal) salts such as potassium phosphites e.g. KH_2PO_3 and K_2HPO_3, sodium phosphites and ammonium phosphites, and (C_1-C_4) alkyl esters of phosphorous acid and their salts such as aluminum ethyl phosphite (fosetyl-Al), calcium ethyl phosphite, magnesium isopropyl phosphite, magnesium isobutyl phosphite, magnesium sec-butyl phosphite and aluminum N-butyl phosphite. Of course, mixtures of phosphite containing compounds are also encompassed. A mixture of e.g. KH_2PO_3 and K_2HPO_3 can easily be obtained by e.g. adding KOH or K_2CO_3 to a final pH of 5.0-6.0 to a KH_2PO_3 solution. As indicated above, precursor-type compounds which in the crop or plant are metabolized into phosphite compounds can also be included in the compositions of the present invention. Examples are phosphonates such as the fosetyl-aluminium complex. In e.g. a crop or plant the ethyl phosphonate part of this molecule is metabolized into a phosphite. An example of such a compound in the commercial ethyl hydrogen phosphonate product called Aliette® (Bayer, Germany). The ratio of phosphite to natamycin (in weight) in the compositions is in general between 2:1 to 500:1 (w/w), preferably between 3:1 to 300:1 (w/w) and more preferably between 5:1 to 200:1 (w/w).

[0019] Compositions according to the invention may have a pH of from 1 to 10, preferably of from 2 to 9, more preferably of from 3 to 8 and most preferably of from 4 to 7. They may be solid, e.g. powder compositions, or may be liquid. The compositions of the present invention can be aqueous or non-aqueous ready-to-use compositions, but may also be aqueous or non-aqueous concentrated compositions/suspensions or stock compositions, suspensions and/or solutions which before use have to be diluted with a suitable diluent such as water or a buffer system. Alternatively, the compositions of the invention can also be used to prepare coating emulsions. The compositions of the present invention can also have the form of concentrated dry products such as e.g. powders, granulates and tablets. They can be used to prepare compositions for immersion or spraying of products such as agricultural products including plants, crops, vegetables and/or fruits. Of course, the above is also applicable when the polyene antifungal compound and the at least one antifungal compound from the family of carboxamide fungicides are applied as separate compositions.

[0020] In a further aspect the invention relates to a kit comprising a polyene antifungal compound and at least one antifungal compound from the family of carboxamide fungicides. The polyene antifungal compound and the at least one antifungal compound from the family of carboxamide fungicides may be present in two separate packages, e.g. containers. The components of the kit may be either in dry form or liquid form in the package. If necessary, the kit may comprise instructions for dissolving the compounds. In addition, the kit may contain instructions for applying the compounds.

[0021] In a further aspect the invention pertains to a method for protecting a product against fungi by treating the product with a polyene antifungal compound and at least one antifungal compound from the family of carboxamide fungicides. In addition, the product can be treated with other antifungal and/or antimicrobial compounds either prior to, concomitant with or after treatment of the products with the polyene antifungal compound and the at least one antifungal compound from the family of carboxamide fungicides. The product may be treated by sequential application of the polyene antifungal compound and the at least one antifungal compound from the family of carboxamide fungicides or vice versa. Alternatively, the product may be treated by simultaneous application of the polyene antifungal compound and the at least one antifungal compound from the family of carboxamide fungicides. In case of simultaneous application, the compounds can be present in different compositions that are applied simultaneously or the compounds may be present in a single composition. In yet another embodiment the product may be treated by separate or alternate modes of applying the antifungal compounds. In an embodiment the invention is

directed to a process for the treatment of products by applying the polyene antifungal compound and the at least one antifungal compound from the family of carboxamide fungicides to the products. By applying the compounds fungal growth on or in the products can be prevented. In other words, the compounds protect the products from fungal growth and/or from fungal infection and/or from fungal spoilage. The compounds can also be used to treat products that have been infected with a fungus. By applying the compounds the disease development due to fungi on or in these products can be slowed down, stopped or the products may even be cured from the disease. In an embodiment of the invention the products are treated with a composition or kit according to the invention. In an embodiment the product is a food, feed, pharmaceutical, cosmetic or agricultural product. In a preferred embodiment the product is an agricultural product.

[0022] The polyene antifungal compound and the at least one antifungal compound from the family of carboxamide fungicides, the compositions according to the invention and the kits according to the invention can be applied to the products by spraying. Other methods suitable for applying these compounds, compositions and kits in liquid form to the products are also a part of the present invention. These include, but are not limited to, dipping, watering, drenching, introduction into a dump tank, vaporizing, atomizing, fogging, fumigating, painting, brushing, dusting, foaming, spreading-on, packaging and coating (e.g. by means of wax or electrostatically). In addition, the antifungal compounds may also be injected into the soil. Spraying applications using automatic systems are known to reduce the labour costs and are cost-effective. Methods and equipment well-known to a person skilled in the art can be used for that purpose. The compositions according to the invention can be regularly sprayed, when the risk of infection is high. When the risk of infection is lower spray intervals may be longer. Depending on the type of application, the amount of polyene antifungal compound applied may vary from 5 ppm to 10,000 ppm, preferably from 10 ppm to 5,000 ppm and most preferably from 20 to 1,000 ppm. Depending on the type of application, the amount of the at least one antifungal compound from the family of carboxamide fungicides applied may vary from 10 ppm to 5,000 ppm, preferably from 20 ppm to 3,000 ppm and most preferably from 50 to 1,000 ppm.

[0023] In a specific embodiment the agricultural product can be treated post-harvest. By using a polyene antifungal compound and the at least one antifungal compound from the family of carboxamide fungicides the control of post-harvest and/or storage diseases is achieved for a long period of time to allow transport of the harvested agricultural product over long distances and under various storage conditions with different controlled atmosphere systems in respect of temperature and humidity. Post-harvest storage disorders are e.g. lenticel spots, scorch, senescent breakdown, bitter pit, scald, water core, browning, vascular breakdown, CO_2 injury, CO_2 or O_2 deficiency, and softening. Fungal diseases may be caused for example by the following fungi: *Blumeria* spp., e.g. *Blumeria graminis; Uncinula* spp., e.g. *Uncinula necator; Leveillula* spp., e.g. *Leveillula taurica; Podosphaera* spp., e.g. *Podosphaera leucotricha, Podosphaera fusca, Podosphaera aphanis; Microsphaera* spp., e.g. *Microsphaera syringae; Sawadaea* spp., e.g. *Sawadaea tulasnei; Mycosphaerella* spp., *Mycosphaerella musae, Mycosphaerella fragariae, Mycosphaerella citri; Mucor* spp., e.g. *Mucor piriformis; Monilinia* spp., e.g. *Monilinia fructigena, Monilinia laxa; Phomopsis* spp., *Phomopsis natalensis; Colletotrichum* spp., e.g. *Colletotrichum musae, Colletotrichum gloeosporioides, Colletotrichum coccodes; Verticillium* spp., e.g. *Verticillium theobromae; Nigrospora* spp.; *Botrytis* spp., e.g. *Botrytis cinerea; Diplodia* spp., e.g. *Diplodia citri; Pezicula* spp.; *Alternaria* spp., e.g. *Alternaria citri, Alternaria alternata; Septoria* spp., e.g. *Septoria depressa; Venturia* spp., e.g. *Venturia inaequalis, Venturia pyrina; Rhizopus* spp., e.g. *Rhizopus stolonifer, Rhizopus oryzae; Glomerella* spp., e.g. *Glomerella cingulata; Sclerotinia* spp., e.g. *Sclerotinia fruiticola; Ceratocystis* spp., e.g. *Ceratocystis paradoxa; Fusarium* spp., e.g. *Fusarium semitectum, Fusarium moniliforme, Fusarium solani, Fusarium oxysporum; Cladosporium* spp., e.g. *Cladosporium fulvum, Cladosporium cladosporioides, Cladosporium cucumerinum, Cladosporium musae; Penicillium* spp., e.g. *Penicillium funiculosum, Penicillium expansum, Penicillium digitatum, Penicillium italicum; Phytophthora* spp., e.g. *Phytophthora citrophthora, Phytophthora fragariae, Phytophthora cactorum, Phytophthora parasitica; Phacydiopycnis* spp., e.g. *Phacydiopycnis malirum; Gloeosporium* spp., e.g. *Gloeosporium album, Gloeosporium perennans, Gloeosporium fructigenum, Gloeosporium singulata; Geotrichum* spp., e.g. *Geotrichum candidum; Phlyctaena* spp., e.g. *Phlyctaena vagabunda; Cylindrocarpon* spp., e.g. *Cylindrocarpon mali; Stemphyllium* spp., e.g. *Stemphyllium vesicarium; Thielaviopsis* spp., e.g. *Thielaviopsis paradoxy; Aspergillus* spp., e.g. *Aspergillus niger, Aspergillus carbonarius; Nectria* spp., e.g. *Nectria galligena; Cercospora* spp., e.g. *Cercospora angreci, Cercospora apii, Cercospora atrofiliformis, Cercospora musae, Cercospora zeae-maydis.*

[0024] Another aspect of the present invention relates to the use of a polyene antifungal compound and at least one antifungal compound from the family of carboxamide fungicides to protect a product against fungi. As indicated above, the compounds may be used, e.g. applied, sequentially or simultaneously. In an embodiment the invention relates to a use, wherein a composition or kit according to the invention is applied to the product. In an embodiment the product is a food, feed, pharmaceutical, cosmetic or agricultural product. In a preferred embodiment the product is an agricultural product.

[0025] In a specific embodiment the polyene antifungal compound and at least one antifungal compound from the family of carboxamide fungicides can be used in medicine, e.g. to treat and/or prevent fungal diseases. The polyene antifungal compound and at least one antifungal compound from the family of carboxamide fungicides can for instance be used in the form of a pharmaceutical composition. The composition may further comprise pharmaceutically acceptable excipients. The antifungal compounds may be administered orally or parenterally. The type of composition is dependent on the route of administration.

[0026] A further aspect of the invention is directed to a product treated with a polyene antifungal compound and at least one antifungal compound from the family of carboxamide fungicides. In an embodiment the product is treated with a composition or kit according to the invention. The invention is therefore directed to a product comprising a polyene antifungal compound and at least one antifungal compound from the family of carboxamide fungicides. The treated products may comprise a polyene antifungal compound and at least one antifungal compound from the family of carboxamide fungicides on their surface and/or inside the product. Alternatively, the treated products may comprise a coating comprising these compounds. In an embodiment the treated products comprise from 0.000001 to 200 mg/dm^2, preferably 0.00001 to 100 mg/dm^2, more preferably from 0.00005 to 10 mg/dm^2 of the polyene antifungal compound on their surface. In a further embodiment they comprise from 0.000001 to 200

mg/dm^2, preferably 0.00001 to 100 mg/dm^2, more preferably from 0.00005 to 10 mg/dm^2 of the at least one antifungal compound from the family of carboxamide fungicides on their surface. In an embodiment the product is a food, feed, pharmaceutical, cosmetic or agricultural product. In a preferred embodiment the product is an agricultural product.

[0027] The term "food products" as used herein is to be understood in a very broad sense and includes, but is not limited to, cheese, cream cheese, shredded cheese, cottage cheese processed cheese, sour cream, dried fermented meat product including salamis and other sausages, wine, beer, yoghurt, juice and other beverages, salad dressing, cottage cheese dressing, dips, bakery products and bakery fillings, surface glazes and icing, spreads, pizza toppings, confectionery and confectionery fillings, olives, olive brine, olive oil, juices, tomato purees and paste, condiments, and fruit pulp and the like food products.

[0028] The term "feed products" as used herein is also to be understood in a very broad sense and includes, but is not limited to, pet food, broiler feed, etc.

[0029] The term "pharmaceutical product" as used herein is also to be understood in a very broad sense and includes products comprising an active molecule such as a drug, agent, or pharmaceutical compound and optionally a pharmaceutically acceptable excipient, i.e. any inert substance that is combined with the active molecule for preparing an agreeable or convenient dosage form.

[0030] The term "cosmetic product" as used herein is also to be understood in a very broad sense and includes products that are used for protecting or treating horny tissues such as skin and lips, hair and nails from drying by preventing transpiration of moisture thereof and further conditioning the tissues as well as giving good appearance to these tissues. Products contemplated by the term "cosmetic product" include, but are not limited to, moisturizers, personal cleansing products, occlusive drug delivery patches, nail polish, powders, wipes, hair conditioners, skin treatment emulsions, shaving creams and the like.

[0031] The term "agricultural products" as used herein is also to be understood in a very broad sense and includes, but is not limited to, cereals, e.g. wheat, barley, rye, oats, rice, sorghum and the like; beets, e.g. sugar beet and fodder beet; pome and stone fruit and berries, e.g. apples, pears, plums, apricots, peaches, almonds, cherries, strawberries, raspberries and blackberries; leguminous plants, e.g. beans, lentils, peas, soy beans; oleaginous plants, e.g. rape, mustard, poppy, olive, sunflower, coconut, castor-oil plant, cocoa, groundnuts; cucurbitaceae, e.g. pumpkins, gherkins, melons, cucumbers, squashes, aubergines; fibrous plants, e.g. cotton, flax, hemp, jute; citrus fruit, e.g. oranges, lemons, grapefruits, mandarins, limes; tropical fruit, e.g. papayas, passion fruit, mangos, carambolas, pineapples, bananas, kiwis; vegetables, e.g. spinach, lettuce, asparagus, brassicaceae such as cabbages and turnips, carrots, onions, tomatoes, potatoes, seedpotatoes, hot and sweet peppers; laurel-like plants, e.g. avocado, cinnamon, camphor tree; or products such as maize, tobacco, nuts, coffee, sugarcane, tea, grapevines, hops, rubber plants, as well as ornamental plants, e.g. cut flowers, roses, tulips, lilies, *narcissus*, crocuses, hyacinths, dahlias, *gerbera*, carnations, fuchsias, chrysanthemums, and flower bulbs, shrubs, deciduous trees and evergreen trees such as conifers, plants and trees in greenhouses. It includes, but is not limited to, plants and their parts, fruits, seeds, cuttings, cultivars, grafts, bulbs, tubers, root-tubers, rootstocks, cut flowers and vegetables.

[0032] A method for preparing a composition as described herein is another aspect of the present invention. The method comprises adding a polyene antifungal compound to at least one antifungal compound from the family of carboxamide fungicides. The compounds may for instance be added separately to an aqueous composition and mixed, followed, if necessary, by adjustment of the pH, viscosity, etc. If added separately, some or all of the separate compounds may be in powder form, but alternatively some or all may also be in liquid form. The compounds may for instance also be added to one another in powder form and mixed to obtain a powdered composition. The powdered composition may then be added to an aqueous composition.

EXAMPLES

Example 1

Treatment of Bananas

[0033] Four organic, unripe (green) bananas are used per treatment. The peel of each banana is wounded thrice using a cork borer according to the method described by de Lapeyre de Bellaire and Dubois (1987). Subsequently, each wound is inoculated with 15 μl of a *Fusarium proliferatum* suspension containing 1×10^5 of spores/ml. After incubation for 4 hours at 20° C., each banana wound is treated with 100 μl of a freshly prepared aqueous antifungal composition comprising either natamycin (DSM Food Specialties, Delft, The Netherlands), boscalid or both. In addition, the carboxamide fungicides carboxin, fenfuram, fenhexamid, furcarbanil, isotianil, methfuroxam, metsulfovax, oxycarboxin, pyracarbolid, thifluzamide and tiadinil alone or in combination with natamycin are tested. The antifungal compositions comprise 1.00% (w/w) methylhydroxyethylcellulose (MHEC), 0.40% (w/w) xanthan gum, 0.20% (w/w) anti-foaming agent, 0.30% (w/w) citric acid, 0.39% (w/w) lactic acid and 0.11% (w/w) potassium sorbate. The pH of the composition is 4.0. A composition without natamycin or a carboxamide fungicide is used as control. The treated, unripe bananas are incubated in a closed box in the dark at 20° C. and a relative air humidity of 95%, which is obtained in the presence of a saturated Na_2HPO_4 aqueous solution. During the first 20 days of incubation, a ripe (yellow) banana is included in the closed box to elevate the ethylene gas level and thus induce ripening of the treated, unripe bananas.

[0034] During incubation, the degree of mould growth on the bananas is assessed in a twofold manner: (i) the number of moulded wounds per total of 12 wounds is counted; and (ii) the antifungal activity (in %) of the individual active ingredients is determined by calculating the reduction in mould growth observed on the banana wounds treated with the antifungal composition in comparison to the mould growth on the banana wounds treated with the control composition. The expected antifungal activity (E in %) of the combined antifungal composition comprising both active ingredients is calculated according to the Colby equation (Colby, 1967):

$$E=X+Y-[(X\cdot Y)/100]$$

wherein X and Y are the observed antifungal activities (in %) of the individual active ingredients X and Y, respectively. If the observed antifungal activity (O in %) of the combination exceeds the expected antifungal activity (E in %) of the combination and the synergy factor O/E is thus >1.0, the combined application of the active ingredients leads to a synergistic antifungal effect.

[0035] The results clearly demonstrate that the antifungal composition comprising both natamycin and a carboxamide fungicide protect bananas better against mould growth than natamycin or a carboxamide fungicide alone.

[0036] Hence, the combination of natamycin and a carboxamide fungicide has synergistic antifungal activity on bananas.

Example 2

Treatment of Strawberries

[0037] Twelve fresh, organic strawberries are used per treatment. Each strawberry is wounded with a 0.5 mm long cut and each wound is inoculated with 10 μl of a *Botrytis cinerea* suspension containing 1×10^5 of spores/ml. After a 2-hour incubation period at 20° C., each strawberry is dipped individually for 1 minute in a freshly prepared aqueous antifungal composition comprising either natamycin (DSM Food Specialties, Delft, The Netherlands), boscalid or both. In addition, the carboxamide fungicides carboxin, fenfuram, fenhexamid, furcarbanil, isotianil, methfuroxam, metsulfovax, oxycarboxin, pyracarbolid, thifluzamide and tiadinil alone or in combination with natamycin are tested. The antifungal compositions also comprise 1.00% (w/w) methylhydroxyethylcellulose (MHEC), 0.40% (w/w) xanthan gum, 0.20% (w/w) anti-foaming agent, 0.30% (w/w) citric acid, 0.39% (w/w) lactic acid and 0.11% (w/w) potassium sorbate. The pH of the composition is 4.0. A composition without natamycin or a carboxamide fungicide is used as control. The treated strawberries are incubated in a closed box in the dark at 20° C.

[0038] After incubation, the mould growth on the strawberries is assessed in a twofold manner: (i) the number of moulded strawberries per total of 12 strawberries is counted; and (ii) the antifungal activity (in %) of the individual and combined active ingredients is determined by calculating the reduction in mould growth observed on the strawberries treated with the antifungal composition in comparison to the mould growth on the strawberries treated with the control composition according to the Colby method described in Example 4 (Colby, 1967).

[0039] The results demonstrate that the antifungal composition comprising natamycin and a carboxamide fungicide have a stronger antifungal activity on strawberries than natamycin or a carboxamide fungicide alone.

[0040] Hence, the combined application of natamycin and a carboxamide fungicide synergistically reduces mould growth on strawberries.

Example 3

Treatment of Mandarins

[0041] Ten fresh, organic mandarins are used per treatment. The peel of each mandarin is wounded once using a cork borer according to the method described by de Lapeyre de Bellaire and Dubois (1987). Subsequently, each wound is inoculated with 10 μl of a *Penicillium italicum* suspension containing 1×10^4 of spores/ml. After incubation for 2 hours at 20° C., the mandarins are dipped individually for 1 minute in a freshly prepared aqueous antifungal composition comprising either natamycin (DSM Food Specialties, Delft, The Netherlands), boscalid or both. In addition, the carboxamide fungicides carboxin, fenfuram, fenhexamid, furcarbanil, isotianil, methfuroxam, metsulfovax, oxycarboxin, pyracarbolid, thifluzamide and tiadinil alone or in combination with natamycin are tested. In addition, the antifungal compositions comprise 3.1% (w/w) beeswax, 0.76% (w/w) glycerol, 0.66% (w/w) polyoxyethylene sorbitan monostearate (Tween 60), 0.03% (w/w) methylhydroxyethylcellulose (MHEC), 0.02% (w/w) xanthan gum, 0.02% (w/w) anti-foaming agent, 0.15% (w/w) citric acid and 0.01% (w/w) potassium sorbate. The pH of the composition is 4.0. A composition without natamycin or a carboxamide fungicide is used as control.

[0042] The treated mandarins are incubated in a closed box in the dark at 20° C. and assessed on mould growth after 25, 28, 31 and 34 days of incubation. The antifungal activity (in %) of the individual and combined active ingredients is determined by calculating the reduction in mould growth observed on the mandarins treated with the antifungal composition in comparison to the mould growth on the mandarins treated with the control composition according to the Colby method (Colby, 1967) described in Example 1 and 2.

[0043] The results prove that the antifungal composition comprising natamycin and a carboxamide fungicide is superior to the compositions comprising natamycin or a carboxamide fungicide alone in preventing mould growth on mandarins.

[0044] Thus, the combined application of natamycin and a carboxamide fungicide synergistically reduces mould growth on mandarins.

Example 4

In Vitro Antifungal Activity

[0045] To demonstrate synergistic antifungal activity of the combination of natamycin with a carboxamide fungicide against *Botrytis cinerea*, an in vitro assay is conducted using 96-well microtiter plates. The following compositions are tested:

[0046] Control (no active ingredient),

[0047] natamycin (DSM Food Specialties, Delft, The Netherlands),

[0048] a carboxamide fungicide,

[0049] natamycin+a carboxamide fungicide.

[0050] After filling each well of a microtiter plate with 92 μl of PCB medium, the active ingredient(s) are added from separate stock solutions prepared in PCB medium or methanol, which resulted in an intermediate volume of 100 μl per well. Subsequently, 100 μl of a *Botrytis cinerea* suspension prepared in PCB medium is used to inoculate each well with 2.5×10^3 spores/ml. Each well thus contains a final volume of 200 μl and <1% of methanol, which does not affect growth of *Botrytis cinerea* (data not shown).

[0051] After incubation of the microtiter plates at 25° C., the in vitro antifungal activity (%) of the individual active ingredients is assessed by calculating the reduction in mould growth observed in the presence of the active ingredient in comparison to the mould growth observed in the absence of the active ingredient. The expected antifungal activity (E in %) of the active ingredient combination is calculated according to the Colby equation (Colby, 1967):

$$E=X+Y-[(X\cdot Y)/100]$$

wherein X and Y are the observed antifungal activities (in %) of the individual active ingredients X and Y, respectively. If the observed antifungal activity (O in %) of the combination exceeds the expected antifungal activity (E in %) of the combination and the resulting synergy factor O/E is thus >1.0, the combined application of the active ingredients leads to a synergistic antifungal effect.

[0052] The results demonstrate that both the natamycin+carboxamide fungicide combination have much stronger antifungal activity against *Botrytis cinerea* than natamycin and a carboxamide fungicide individually.

[0053] Hence, the combined application of natamycin and a carboxamide fungicide synergistically inhibits growth of *Botrytis cinerea*.

Example 5

Treatment of Strawberries

[0054] Twelve fresh, organic strawberries were used per treatment. Each strawberry was wounded with a 0.5 mm long cut and each wound was inoculated with 10 μl of a *Botrytis cinerea* suspension containing 1×10^5 of spores/ml. After a 3-hour incubation period at 20° C., each strawberry was dipped individually for 1 minute in a freshly prepared aqueous antifungal composition comprising either 500 ppm natamycin (DSM Food Specialties, Delft, The Netherlands), 500 ppm fenhexamid or both. Each antifungal composition also comprised 3.2% (w/w) beeswax, 0.8% (w/w) glycerol, 0.7% (w/w) polyoxyethylene sorbitan monostearate (Tween 60), 0.1% (w/w) polyoxyethylene sorbitan monooleate (Tween 80), 0.05% (w/w) methylhydroxyethyl-cellulose (MHEC), 0.03% (w/w) anti-foaming agent, 0.02% (w/w) xanthan gum, 0.02% (w/w) citric acid, 0.01% (w/w) lactic acid and 0.01% potassium sorbate. A composition without natamycin or fenhexamid was used as control. Each composition had a pH of 4. The treated strawberries were incubated in a closed box in the dark at 20° C. for 11 days.

[0055] During incubation, mould growth on the strawberries was assessed in a twofold manner: (i) the number of moulded strawberries per total of 12 strawberries was counted; and (ii) the antifungal activity (in %) of the individual and combined active ingredients was determined by calculating the reduction in mould growth observed on the strawberries treated with the antifungal composition in comparison to the mould growth on the strawberries treated with the control composition. The expected antifungal activity (E in %) of the combined antifungal composition comprising both active ingredients was calculated according to the Colby equation (Colby, 1967):

$$E=X+Y-[(X\cdot Y)/100]$$

wherein X and Y are the observed antifungal activities (in %) of the individual active ingredients X and Y, respectively. If the observed antifungal activity (O in %) of the combination exceeds the expected antifungal activity (E in %) of the combination and the synergy factor O/E is thus >1.0, the combined application of the active ingredients leads to a synergistic antifungal effect.

[0056] The results in Table 1 (number of moulded strawberries per total of 12 strawberries) and Table 2 (antifungal activity) unequivocally demonstrate that the combined antifungal composition comprising 500 ppm natamycin and 500 ppm fenhexamid protected strawberries more effectively against mould growth than the compositions comprising natamycin or fenhexamid alone.

[0057] After 6 through 9 days of incubation, all 12 strawberries treated with either the control composition, natamycin alone or fenhexamid alone were moulded. However, of the 12 strawberries treated with the active ingredient combination of natamycin and fenhexamid, only 5, 7, 7, and 8 were moulded after 6, 7, 8 and 9 days of incubation, respectively (see Table 1).

[0058] Moreover, the observed antifungal activity exceeded the expected antifungal activity with approximately 10 to >60% between 4 and 11 days of incubation, which yielded synergy factors ranging from 1.9 to 13 (see Table 2).

[0059] Thus, the combined application of 500 ppm natamycin and 500 ppm fenhexamid leads to a surprisingly strong synergistic reduction in mould growth on strawberries.

Example 6

Treatment of Strawberries

[0060] The experiment was conducted as described in Example 5, except for the fact that each wounded and inoculated strawberry was dipped individually for 1 minute in a freshly prepared aqueous antifungal composition comprising either 250 ppm natamycin (DSM Food Specialties, Delft, The Netherlands), 250 ppm fenhexamid or both. The treated strawberries were incubated in a closed box in the dark at 20° C. for 10 days. During incubation, the treated strawberries were assessed on mould growth according to the two methods described in Example 5.

[0061] The results in Table 3 (number of moulded strawberries per total of 12 strawberries) and Table 4 (antifungal activity) clearly demonstrate that the antifungal composition comprising 250 ppm natamycin and 250 ppm fenhexamid had a much stronger antifungal effect on strawberries than natamycin or fenhexamid alone.

[0062] After 5 days of incubation, all 12 strawberries treated with the control composition showed mould growth, whereas 9 of the 12 strawberries treated with natamycin alone and 11 of the 12 strawberries treated with fenhexamid alone were moulded. However, mould growth was observed only for 7 of the 12 strawberries treated with the composition comprising natamycin and fenhexamid (see Table 3).

[0063] After 6 and 7 days of incubation, all 12 strawberries treated with the control composition were moulded, as were 11 of the 12 strawberries treated with either natamycin alone or fenhexamid alone. However, only 9 of the 12 strawberries treated with the composition comprising natamycin and fenhexamid were moulded (see Table 3).

[0064] After 8 days of incubation, all 12 strawberries treated with either the control composition or fenhexamid alone showed mould growth, as did 11 of the 12 strawberries treated with natamycin alone. However, only 9 of the 12 strawberries treated with both natamycin and fenhexamid were moulded (see Table 3).

[0065] Moreover, the observed antifungal activity was 6 to about 12% higher than the expected antifungal activity between 8 and 10 days of incubation. Consequently, the corresponding synergy factor exceeded 1.0 and increased from 1.3 on day 8 to 3.5 on day 10 (see Table 4).

[0066] Hence, the combined application of 250 ppm natamycin and 250 ppm fenhexamid has a synergistic antifungal effect on strawberries.

Example 7

Treatment of Oranges

[0067] Ten fresh, organic oranges were used per treatment. Each orange was soaked in a 180 ppm hypochlorite solution for 10 minutes, then rinsed thoroughly with fresh tap water and dried. The peel of each disinfected orange was wounded once using a cork borer according to the method described by de Lapeyre de Bellaire and Dubois (1987). Subsequently, each wound was inoculated with 10 μl of a *Penicillium italicum* suspension containing 1×10^5 of spores/ml. After incubation for 3 hours at 20° C., each wound and the orange peel area of 1 cm around the wound was treated with in total 150 μl of a freshly prepared aqueous antifungal composition comprising either 500 ppm natamycin (DSM Food Specialties, Delft, The Netherlands), 800 ppm boscalid or both. Each antifungal composition also comprised 3.2% (w/w) beeswax, 0.8% (w/w) glycerol, 0.7% (w/w) polyoxyethylene sorbitan monostearate (Tween 60), 0.2% (w/w) polyoxyethylene sor-

bitan monooleate (Tween 80), 0.05% (w/w) methylhydroxyethyl-cellulose (MHEC), 0.03% (w/w) anti-foaming agent, 0.02% (w/w) xanthan gum, 0.02% (w/w) citric acid, 0.01% (w/w) lactic acid and 0.01% potassium sorbate. A composition without natamycin or boscalid was used as control. Each composition had a pH of 4. The treated oranges were incubated in a closed box in the dark at 20° C. and assessed on mould growth during a 17-day incubation period. The antifungal activity (in %) of the individual and combined active ingredients was determined by calculating the reduction in mould growth observed on the oranges treated with the antifungal composition in comparison to the mould growth on the oranges treated with the control composition according to the Colby method (Colby, 1967) described in Example 5.

[0068] The results in Table 5 reveal that the active ingredient combination of 500 ppm natamycin and 800 ppm boscalid was more successful in limiting mould growth on oranges than natamycin or boscalid alone.

[0069] After 7 through 17 days of incubation, the observed antifungal activity of the composition comprising natamycin and boscalid was 5 to nearly 60% higher than the expected antifungal activity. As a result, the corresponding synergy factor increased from 1.1 on day 7 to 3.6 on day 17 (see Table 5).

[0070] In conclusion, the results of this example clearly demonstrate the synergistic antifungal effect of 500 ppm natamycin and 800 ppm boscalid when applied in combination on oranges.

Example 8

Treatment of Oranges

[0071] The experiment was conducted as described in Example 7, except for the fact that each wounded and inoculated orange was treated with 150 μl of a freshly prepared aqueous antifungal composition comprising either 250 ppm natamycin (DSM Food Specialties, Delft, The Netherlands), 400 ppm boscalid or both. The treated oranges were incubated in a closed box in the dark at 20° C. and assessed on mould growth during a 18-day incubation period. During incubation, the treated oranges were assessed on mould growth according to the method described in Example 7.

[0072] The results in Table 6 show the higher antifungal activity of the composition comprising 250 ppm natamycin and 400 ppm boscalid compared to the antifungal activities of the compositions comprising natamycin or boscalid alone.

[0073] The observed antifungal activity of the active ingredient combination of natamycin and boscalid exceeded the expected antifungal activity with 7 to >20% between day 11 and 18. Consequently, the synergy factor was always >1.0 and increased from 1.1 on day 11 to 1.6 on day 18 (see Table 6).

[0074] Thus, this example proves the synergistic antifungal effect of the combined application of 250 ppm natamycin and 400 ppm boscalid on oranges.

Example 9

Treatment of Sweet Peppers

[0075] Ten fresh, organic sweet peppers were used per treatment. Each sweet pepper was soaked in a 180 ppm hypochlorite solution for 10 minutes, then rinsed thoroughly with fresh tap water and dried. The peel of each disinfected sweet pepper was wounded once using a cork borer according to the method described by de Lapeyre de Bellaire and Dubois (1987). Subsequently, each wound was inoculated with 10 μl of a *Botrytis cinerea* suspension containing 1×10^5 of spores/ml. After incubation for 3 hours at 20° C., each wound and the skin area of 0.5 cm around the wound was treated with in total 75 μl of a freshly prepared aqueous antifungal composition comprising either 400 ppm natamycin (DSM Food Specialties, Delft, The Netherlands), 600 ppm fenhexamid or both. Each antifungal composition also comprised 3.2% (w/w) beeswax, 0.8% (w/w) glycerol, 0.7% (w/w) polyoxyethylene sorbitan monostearate (Tween 60), 0.2% (w/w) polyoxyethylene sorbitan monooleate (Tween 80), 0.05% (w/w) methylhydroxyethyl-cellulose (MHEC), 0.03% (w/w) anti-foaming agent, 0.02% (w/w) xanthan gum, 0.02% (w/w) citric acid, 0.01% (w/w) lactic acid and 0.01% potassium sorbate. A composition without natamycin or fenhexamid was used as control. Each composition had a pH of 4.

[0076] The treated sweet peppers were incubated in a closed box in the dark at 20° C. and assessed on mould growth during a 26-day incubation period. The antifungal activity (in %) of the individual and combined active ingredients was determined by calculating the reduction in mould growth observed on the sweet peppers treated with the antifungal composition in comparison to the mould growth on the sweet peppers treated with the control composition according to the Colby method (Colby, 1967) described in Example 5.

[0077] The results in Table 7 show that the combined antifungal composition comprising 400 ppm natamycin and 600 ppm fenhexamid protected sweet peppers more effectively against mould growth than the compositions comprising either natamycin or fenhexamid.

[0078] The observed antifungal activity exceeded the expected antifungal activity with approximately 5 to 14% between 6 and 26 days of incubation, which yielded synergy factors >1.0 (see Table 7).

[0079] Thus, the combined application of 400 ppm natamycin and 600 ppm fenhexamid synergistically reduces mould growth on sweet peppers.

Example 10

Treatment of Sweet Peppers

[0080] The experiment was conducted as described in Example 9, except for the fact that each wounded and inoculated sweet pepper was treated with 75 μl of a freshly prepared aqueous antifungal composition comprising either 400 ppm natamycin (DSM Food Specialties, Delft, The Netherlands), 800 ppm carboxin or both. The treated sweet peppers were incubated in a closed box in the dark at 20° C. and assessed on mould growth during a 26-day incubation period. During incubation, the treated sweet peppers were assessed on mould growth according to the method described in Example 9.

[0081] The results in Table 8 demonstrate that the antifungal composition comprising both 400 ppm natamycin and 800 ppm carboxin had a stronger antifungal effect on sweet peppers than natamycin or carboxin alone.

[0082] After 17 through 26 days of incubation, the observed antifungal activity was approximately 5 to 8% higher than the expected antifungal activity (see Table 8). Consequently, synergy factors >1.0 were obtained.

[0083] In conclusion, synergistic antifungal activity exists between 400 ppm natamycin and 800 ppm carboxin when applied in combination on sweet peppers.

TABLE 1

Number of moulded strawberries incubated at 20° C. after treatment with compositions comprising either 500 ppm natamycin, 500 ppm fenhexamid or both.

Antifungal composition	Number of moulded strawberries/ total number of 12 strawberries during incubation time (in days) Day 6	Day 7-8	Day 9
Control	12/12	12/12	12/12
Natamycin 500 ppm	12/12	12/12	12/12
Fenhexamid 500 ppm	12/12	12/12	12/12
Natamycin 500 ppm + fenhexamid 500 ppm	5/12	7/12	8/12

TABLE 2

Antifungal activity (%) of compositions comprising either 500 ppm natamycin, 500 ppm fenhexamid or both on strawberries after incubation at 20° C.

Antifungal composition	Incubation time (days)	Observed antifungal activity O (%)	Expected antifungal activity E (%)	Synergy factor O/E
Control	4	0	—	—
Natamycin 500 ppm		33	—	—
Fenhexamid 500 ppm		22	—	—
Natamycin 500 ppm + fenhexamid 500 ppm		89	48	1.9
Control	5	0	—	—
Natamycin 500 ppm		16	—	—
Fenhexamid 500 ppm		25	—	—
Natamycin 500 ppm + fenhexamid 500 ppm		77	37	2.1
Control	6	0	—	—
Natamycin 500 ppm		5.3	—	—
Fenhexamid 500 ppm		0	—	—
Natamycin 500 ppm + fenhexamid 500 ppm		68	5.3	13
Control	7	0	—	—
Natamycin 500 ppm		11	—	—
Fenhexamid 500 ppm		0	—	—
Natamycin 500 ppm + fenhexamid 500 ppm		54	11	4.9
Control	8	0	—	—

TABLE 2-continued

Antifungal activity (%) of compositions comprising either 500 ppm natamycin, 500 ppm fenhexamid or both on strawberries after incubation at 20° C.

Antifungal composition	Incubation time (days)	Observed antifungal activity O (%)	Expected antifungal activity E (%)	Synergy factor O/E
Natamycin 500 ppm		14	—	—
Fenhexamid 500 ppm		0	—	—
Natamycin 500 ppm + fenhexamid 500 ppm		53	14	3.8
Control	9	0	—	—
Natamycin 500 ppm		4.8	—	—
Fenhexamid 500 ppm		0	—	—
Natamycin 500 ppm + fenhexamid 500 ppm		49	4.8	10
Control	10	0	—	—
Natamycin 500 ppm		2.4	—	—
Fenhexamid 500 ppm		0	—	—
Natamycin 500 ppm + fenhexamid 500 ppm		25	2.4	10
Control	11	0	—	—
Natamycin 500 ppm		2.4	—	—
Fenhexamid 500 ppm		0	—	—
Natamycin 500 ppm + fenhexamid 500 ppm		13	2.4	5.4

TABLE 3

Number of moulded strawberries incubated at 20° C. after treatment with compositions comprising either 250 ppm natamycin, 250 ppm fenhexamid or both.

Antifungal composition	Number of moulded strawberries/ total number of 12 strawberries during incubation time (in days) Day 5	Day 6-7	Day 8
Control	12/12	12/12	12/12
Natamycin 250 ppm	9/12	11/12	11/12
Fenhexamid 250 ppm	11/12	11/12	12/12
Natamycin 250 ppm + fenhexamid 250 ppm	7/12	9/12	9/12

TABLE 4

Antifungal activity (%) of compositions comprising either 250 ppm natamycin, 250 ppm fenhexamid or both on strawberries after incubation at 20° C.

Antifungal composition	Incubation time (days)	Observed antifungal activity O (%)	Expected antifungal activity E (%)	Synergy factor O/E
Control	8	0	—	—
Natamycin 250 ppm		18	—	—
Fenhexamid 250 ppm		7.5	—	—
Natamycin 250 ppm + fenhexamid 250 ppm		30	24	1.3
Control	9	0	—	—
Natamycin 250 ppm		13	—	—
Fenhexamid 250 ppm		0	—	—
Natamycin 250 ppm + fenhexamid 250 ppm		21	13	1.6
Control	10	0	—	—
Natamycin 250 ppm		4.8	—	—
Fenhexamid 250 ppm		0	—	—
Natamycin 250 ppm + fenhexamid 250 ppm		17	4.8	3.5

TABLE 5

Antifungal activity (%) of compositions comprising either 500 ppm natamycin, 800 ppm boscalid or both on oranges after incubation at 20° C.

Antifungal composition	Incubation time (days)	Observed antifungal activity O (%)	Expected antifungal activity E (%)	Synergy factor O/E
Control	7	0	—	—
Natamycin 500 ppm		89	—	—
Boscalid 800 ppm		54	—	—
Natamycin 500 ppm + boscalid 800 ppm		100	95	1.1
Control	8	0	—	—
Natamycin 500 ppm		86	—	—
Boscalid 800 ppm		40	—	—
Natamycin 500 ppm + boscalid 800 ppm		100	91	1.1
Control	9	0	—	—
Natamycin 500 ppm		64	—	—
Boscalid 800 ppm		39	—	—
Natamycin 500 ppm + boscalid 800 ppm		100	78	1.3
Control	10	0	—	—
Natamycin 500 ppm		54	—	—
Boscalid 800 ppm		29	—	—
Natamycin 500 ppm + boscalid 800 ppm		100	68	1.5
Control	11	0	—	—
Natamycin 500 ppm		41	—	—
Boscalid 800 ppm		35	—	—
Natamycin 500 ppm + boscalid 800 ppm		97	62	1.6
Control	12	0	—	—
Natamycin 500 ppm		36	—	—
Boscalid 800 ppm		18	—	—
Natamycin 500 ppm + boscalid 800 ppm		95	48	2.0
Control	13	0	—	—
Natamycin 500 ppm		38	—	—
Boscalid 800 ppm		20	—	—
Natamycin 500 ppm + boscalid 800 ppm		91	50	1.8
Control	14	0	—	—
Natamycin 500 ppm		32	—	—
Boscalid 800 ppm		8	—	—

TABLE 5-continued

Antifungal activity (%) of compositions comprising either 500 ppm natamycin, 800 ppm boscalid or both on oranges after incubation at 20° C.

Antifungal composition	Incubation time (days)	Observed antifungal activity O (%)	Expected antifungal activity E (%)	Synergy factor O/E
Natamycin 500 ppm + boscalid 800 ppm		91	38	2.4
Control	15	0	—	—
Natamycin 500 ppm		24	—	—
Boscalid 800 ppm		8	—	—
Natamycin 500 ppm + boscalid 800 ppm		88	30	2.9
Control	17	0	—	—
Natamycin 500 ppm		21	—	—
Boscalid 800 ppm		0	—	—
Natamycin 500 ppm + boscalid 800 ppm		76	21	3.6

TABLE 6

Antifungal activity (%) of compositions comprising either 250 ppm natamycin, 400 ppm boscalid or both on oranges after incubation at 20° C.

Antifungal composition	Incubation time (days)	Observed antifungal activity O (%)	Expected antifungal activity E (%)	Synergy factor O/E
Control	11	0	—	—
Natamycin 250 ppm		69	—	—
Boscalid 400 ppm		35	—	—
Natamycin 250 ppm + boscalid 400 ppm		87	80	1.1
Control	12	0	—	—
Natamycin 250 ppm		66	—	—
Boscalid 400 ppm		22	—	—
Natamycin 250 ppm + boscalid 400 ppm		83	73	1.1
Control	13	0	—	—
Natamycin 250 ppm		62	—	—
Boscalid 400 ppm		24	—	—
Natamycin 250 ppm + boscalid 400 ppm		81	71	1.1
Control	14	0	—	—
Natamycin 250 ppm		51	—	—
Boscalid 400 ppm		15	—	—
Natamycin 250 ppm + boscalid 400 ppm		74	58	1.3
Control	15	0	—	—
Natamycin 250 ppm		46	—	—
Boscalid 400 ppm		10	—	—
Natamycin 250 ppm + boscalid 400 ppm		72	51	1.4
Control	17	0	—	—
Natamycin 250 ppm		39	—	—
Boscalid 400 ppm		6	—	—
Natamycin 250 ppm + boscalid 400 ppm		60	42	1.4
Control	18	0	—	—
Natamycin 250 ppm		34	—	—
Boscalid 400 ppm		1	—	—
Natamycin 250 ppm + boscalid 400 ppm		55	35	1.6

TABLE 7

Antifungal activity (%) of compositions comprising either 400 ppm natamycin, 600 ppm fenhexamid or both on sweet peppers after incubation at 20° C.

Antifungal composition	Incubation time (days)	Observed antifungal activity O (%)	Expected antifungal activity E (%)	Synergy factor O/E
Control	6	0	—	—
Natamycin 400 ppm		95	—	—
Fenhexamid 600 ppm		0	—	—
Natamycin 400 ppm + Fenhexamid 600 ppm		100	95	1.1
Control	8	0	—	—
Natamycin 400 ppm		94	—	—
Fenhexamid 600 ppm		0	—	—
Natamycin 400 ppm + Fenhexamid 600 ppm		100	94	1.1
Control	10	0	—	—
Natamycin 400 ppm		89	—	—
Fenhexamid 600 ppm		00	—	—
Natamycin 400 ppm + Fenhexamid 600 ppm		100	89	1.1
Control	11	0	—	—
Natamycin 400 ppm		87	—	—
Fenhexamid 600 ppm		0	—	—
Natamycin 400 ppm + Fenhexamid 600 ppm		100	87	1.1
Control	13-17	0	—	—
Natamycin 400 ppm		86	—	—
Fenhexamid 600 ppm		0	—	—
Natamycin 400 ppm + Fenhexamid 600 ppm		100	86	1.2
Control	18-22	0	—	—
Natamycin 400 ppm		86	—	—
Fenhexamid 600 ppm		0	—	—
Natamycin 400 ppm + Fenhexamid 600 ppm		98	86	1.1
Control	24	0	—	—
Natamycin 400 ppm		83	—	—
Fenhexamid 600 ppm		0	—	—
Natamycin 400 ppm + Fenhexamid 600 ppm		97	83	1.2
Control	25	0	—	—
Natamycin 400 ppm		82	—	—
Fenhexamid 600 ppm		0	—	—
Natamycin 400 ppm + Fenhexamid 600 pp		95	82	1.2
Control	26	0	—	—
Natamycin 400 ppm		79	—	—
Fenhexamid 600 ppm		0	—	—
Natamycin 400 ppm + Fenhexamid 600 ppm		93	79	1.2

TABLE 8

Antifungal activity (%) of compositions comprising either 400 ppm natamycin, 800 ppm carboxin or both on sweet peppers after incubation at 20° C.

Antifungal composition	Incubation time (days)	Observed antifungal activity O (%)	Expected antifungal activity E (%)	Synergy factor O/E
Control	17-19	0	—	—
Natamycin 400 ppm		86	—	—
Carboxin 800 ppm		67	—	—
Natamycin 400 ppm + Carboxin 800 ppm		100	95	1.1
Control	20-21	0	—	—
Natamycin 400 ppm		87	—	—
Carboxin 800 ppm		55	—	—

TABLE 8-continued

Antifungal activity (%) of compositions comprising either 400 ppm natamycin, 800 ppm carboxin or both on sweet peppers after incubation at 20° C.

Antifungal composition	Incubation time (days)	Observed antifungal activity O (%)	Expected antifungal activity E (%)	Synergy factor O/E
Natamycin 400 ppm + Carboxin 800 ppm		100	94	1.1
Control	22	0	—	—
Natamycin 400 ppm		85	—	—
Carboxin 800 ppm		45	—	—
Natamycin 400 ppm + Carboxin 800 ppm		98	91	1.1
Control	24	0	—	—
Natamycin 400 ppm		83	—	—
Carboxin 800 ppm		42	—	—
Natamycin 400 ppm + Carboxin 800 ppm		98	90	1.1
Control	25	0	—	—
Natamycin 400 ppm		82	—	—
Carboxin 800 ppm		41	—	—
Natamycin 400 ppm + Carboxin 800 ppm		94	89	1.1
Control	26	0	—	—
Natamycin 400 ppm		79	—	—
Carboxin 800 ppm		38	—	—
Natamycin 400 ppm + Carboxin 800 ppm		93	87	1.1

REFERENCES

[0084] Avenot H F and Michailides T J (2007), Resistance to boscalid fungicide in *Alternaria alternata* isolates from Pistachio in California. Plant Disease 91: 1345-1350.

[0085] Colby S R (1967), Calculating synergistic and antagonistic responses of herbicide combination. Weeds 15: 20-22.

[0086] Fillinger S, Leroux P, Auclair C, Barreau C, Al Hajj C and Debieu D (2008), Genetic analysis of fenhexamid-resistant field isolates of the phytopathogenic fungus *Botrytis cinerea*. Antimicrob. Agents Chemother. 52: 3933-3940.

[0087] Lapeyre de Bellaire de L and Dubois C (1987), Distribution of Thiabendazole-Resistant *Colletotrichum musae* Isolates from Guadeloupe Banana Plantations. Plant Disease 81:1378-1383.

[0088] Leroux P and Berthier G (1988), Resistance to carboxin and fenfuram in *Ustilago nuda* (Jens.) Rostr., the causal agent of barley loose smut. Crop Protection 7: 16-19.

[0089] Slinker B K (1998), The Statistics of Synergism. Journal of Mol. and Cell. Cardiology 30:723-731.

1. A composition comprising a polyene antifungal compound and at least one antifungal compound from the family of carboxamide fungicides.

2. A composition according to claim **1**, wherein the at least one antifungal compound from the family of carboxamide fungicides is selected from the group consisting of boscalid, carboxin, fenfuram, fenhexamid, furcarbanil, isotianil, methfuroxam, metsulfovax, oxycarboxin, pyracarbolid, thifluzamide and tiadinil.

3. A composition according to claim **1**, wherein the polyene antifungal compound is natamycin.

4. A composition according to claim **1**, wherein the composition further comprises at least one additional compound selected from the group consisting of a sticking agent, a

carrier, a colouring agent, a protective colloid, an adhesive, a herbicide, a fertilizer, a thickening agent, a sequestering agent, a thixotropic agent, a surfactant, a further antimicrobial compound, a detergent, a preservative, a spreading agent, a filler, a spray oil, a flow additive, a mineral substance, a solvent, a dispersant, an emulsifier, a wetting agent, a stabiliser, an antifoaming agent, a buffering agent, an UV-absorber and an antioxidant.

5. A composition according to claim **1**, wherein the amount of the polyene antifungal compound is in the range from 0.005 g/l to about 100 g/l and the amount of the at least one antifungal compound from the family of carboxamide fungicides is in the range from about 0.0001 g/l to about 2000 g/l.

6. A kit comprising a polyene antifungal compound and at least one antifungal compound from the family of carboxamide fungicides.

7. A method for protecting a product against fungi by treating the product with a polyene antifungal compound and at least one antifungal compound from the family of carboxamide fungicides.

8. A method according to claim **7**, wherein the product is treated with a composition according to claim **1**.

9. A method according to claim **7**, wherein the product is selected from the group consisting of a food product, a feed product, a pharmaceutical product, a cosmetic product and an agricultural product.

10. A method according to claim **9**, wherein the product is an agricultural product.

11. A method according to claim **10**, wherein the product is treated post-harvest.

12. A product comprising a polyene antifungal compound and at least one antifungal compound from the family of carboxamide fungicides.

13. A product according to claim **12**, wherein the product is selected from the group consisting of a food product, a feed product, a pharmaceutical product, a cosmetic product and an agricultural product.

14. A product according to claim **13**, wherein the product is an agricultural product.

15. A polyene antifungal compound and at least one antifungal compound from the family of carboxamide fungicides capable of being used to protect a product against fungi.

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