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(54) Title: NEW ANTIFUNGAL COMPOSITIONS

(57) Abstract: The present invention relates to new antifungal compositions and their use in the treatment of agricultural products.

NEW ANTIFUNGAL COMPOSITIONS

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Field of the invention

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

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Background of the invention

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.

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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.

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An important class of fungicides are the strobilurins. They were first sold in 1996. Sales of strobilurins and related compounds totalled about \$ 1500 million in 2004 which representing over 20% of the global fungicide market. Strobilurins are used in for instance cereals, turf grass, grapevines, potatoes and fruit, nut and vegetable crops. The huge impact of the strobilurin family on agriculture is well-reflected by the current status of azoxystrobin, which is registered for use on more than 80 different crops in more than 70 countries, representing over \$ 500 million sales in 2004. It has also been suggested to use azoxystrobin as an anti-mould agent for the preservation of foodstuff (see WO 02/00027). Although the strobilurin fungicides have shown activity against fungi (see WO 2007/104677, WO 2007/104669, US 7,071,188; US 7,098,170 and US 7,807,714), spoilage problems still occur. Moreover, several studies have shown that more and more fungi acquire resistance against these fungicides (see Heaney *et al.*, 2000; Sierotzki *et al.*, 2000; Bartlett *et al.*, 2002). Furthermore, some of the currently used strobilurins such

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as e.g. metominostrobin have the disadvantage of being dangerous for the health of exposed persons and the environment (see Tomlin, 2000).

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
5 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 is applied for many years in e.g. the cheese industry, up to now development of resistant fungal species was never observed.

10 Consequently, it can be concluded that there is a severe need for more effective, more environmental friendly, lower-toxicity and less harmful antimicrobial compositions, e.g. antifungal compositions, for the treatment of fungal growth in and on plants and crops.

15 Description of the invention

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 strobilurin fungicides. As used
20 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.

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:
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$$\frac{((\text{value of evolution status of untreated control} - \text{value of evolution status of composition}) / (\text{value of evolution status of untreated control})) * 100}{((\text{relative efficacy of combination compound A} + \text{compound B}) / (\text{relative efficacy of compound A} + \text{relative efficacy of compound B})) * 100}$$
An interaction coefficient larger than 100 indicates synergy
30 between the compounds.

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.

In an embodiment of the invention the composition according to the invention comprises at least one antifungal compound from the family of strobilurin fungicides selected from the group consisting of azoxystrobin, bifujunzhi, coumoxystrobin, dimoxystrobin, enestroburin, fluoxastrobin, jiaxiangjunzhi, kresoxim-methyl, lvdinjunzhi, metominostrobin, orysastrobin, picoxystrobin, pyraclostrobin, pyrametostrobin, pyraoxystrobin, trifloxystrobin, and xiwojunan. In a preferred embodiment the composition comprises at least one antifungal compound from the family of strobilurin fungicides selected from the group consisting of azoxystrobin, dimoxystrobin, fluoxastrobin, kresoxim-methyl, metominostrobin, orysastrobin, picoxystrobin, pyraclostrobin and trifloxystrobin. In an even more preferred embodiment the composition comprises at least one antifungal compound from the family of strobilurin fungicides selected from the group consisting of azoxystrobin, fluoxastrobin, kresoxim-methyl, picoxystrobin, pyraclostrobin and trifloxystrobin.

In an embodiment the compositions may also contain two or more different antifungal compounds from the family of strobilurin fungicides. It is to be understood that derivatives of antifungal compounds from the family of strobilurin fungicides including, but not limited to, salts or solvates of antifungal compounds from the family of strobilurin fungicides or modified forms of antifungal compounds from the family of strobilurin fungicides may also be applied in the compositions of the invention. Examples of commercial products containing strobilurin fungicides such as azoxystrobin are the products with the brand name Abound® (azoxystrobin), Amistar® (azoxystrobin), Dynasty® (azoxystrobin + metalaxyl-M + fludioxonil), Heritage® (azoxystrobin) or Quadris® (azoxystrobin). Examples of commercial products containing strobilurin fungicides such as trifloxystrobin are the products with the brand name Flint® (trifloxystrobin), Gem® (trifloxystrobin), Compass® (trifloxystrobin) or Trilex® (trifloxystrobin). Examples of commercial products containing strobilurin fungicides such as fluoxastrobin are the products with the brand name Bariton® (fluoxastrobin +

prothioconazole), EFA® (fluoxastrobin + prothioconazole + tebuconazole + triazoxide) or Scenic® (fluoxastrobin + prothioconazole + tebuconazole). Examples of commercial products containing strobilurin fungicides such as pyraclostrobin are the products with the brand name Cabrio® (pyraclostrobin), Opera® (pyraclostrobin + epoxiconazole) or Comet® (pyraclostrobin). Examples of commercial products containing strobilurin fungicides such as picoxystrobin are the products with the brand name Acanto® (picoxystrobin), Furlong® (picoxystrobin + cyproconazole) or Credo® (picoxystrobin + chlorothalonil). Examples of other commercial products containing strobilurin fungicides are the products with the brand name Imochi Ace® (metominostrobin), Wullingshi® (coumoxystrobin), Swing Gold® (dimoxystrobin + epoxiconazole), Sovran® (kresoxim-methyl) or Agro® (orysastrobin). Said commercial products can be incorporated in the present invention.

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 Delvacid®. 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.

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.

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 strobilurin fungicides. More preferably, the amount is from 0.001 g/l to 1000 g/l.

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 chlorthalonil) 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 strobilurin 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.

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).

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 strobilurin fungicides are applied as separate compositions.

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 strobilurin fungicides. The polyene antifungal compound and the at least one antifungal compound from the family of strobilurin 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. In an embodiment of the invention the kit according to the invention comprises at least one antifungal compound from the family of strobilurin fungicides selected from the group consisting of azoxystrobin, bifujunzhi, coumoxystrobin, dimoxystrobin, enestroburin, fluoxastrobin,

jiaxiangjunzhi, kresoxim-methyl, lvdngjunzhi, metominostrobin, oryastrobin, picoxystrobin, pyraclostrobin, pyrametostrobin, pyraoxystrobin, trifloxystrobin, and xiwojunan. In a preferred embodiment the kit comprises at least one antifungal compound from the family of strobilurin fungicides selected from the group consisting of azoxystrobin, dimoxystrobin, fluoxastrobin, kresoxim-methyl, metominostrobin, oryastrobin, picoxystrobin, pyraclostrobin and trifloxystrobin. In an even more preferred embodiment the kit comprises at least one antifungal compound from the family of strobilurin fungicides selected from the group consisting of azoxystrobin, fluoxastrobin, kresoxim-methyl, picoxystrobin, pyraclostrobin and trifloxystrobin.

In a further aspect the invention pertains to a method for protecting a product against fungi by treating the agricultural product with a polyene antifungal compound and at least one antifungal compound from the family of strobilurin 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 strobilurin 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 strobilurin 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 strobilurin 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 at least one antifungal compound from the family of strobilurin fungicides is selected from the group consisting of azoxystrobin, bifujunzhi, coumoxystrobin, dimoxystrobin, enestroburin, fluoxastrobin, jiaxiangjunzhi, kresoxim-methyl, lvdngjunzhi, metominostrobin, oryastrobin, picoxystrobin, pyraclostrobin, pyrametostrobin, pyraoxystrobin, trifloxystrobin, and xiwojunan. In a preferred embodiment the at least one antifungal compound from the family of strobilurin fungicides is selected from the group consisting of azoxystrobin, dimoxystrobin, fluoxastrobin, kresoxim-methyl, metominostrobin, oryastrobin, picoxystrobin, pyraclostrobin and trifloxystrobin. In an even more preferred embodiment the at least one antifungal compound from the family of strobilurin fungicides selected from the group consisting of azoxystrobin, fluoxastrobin, kresoxim-methyl, picoxystrobin,

pyraclostrobin and trifloxystrobin. 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 strobilurin 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.

The polyene antifungal compound and the at least one antifungal compound from the family of strobilurin 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 compounds and compositions 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 strobilurin 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.

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 strobilurin 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, CO₂ injury, CO₂ or O₂ deficiency, and softening. Fungal diseases may be caused for example by the following fungi: *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 atrofiformis*, *Cercospora musae*, *Cercospora zeae-maydis*.

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 strobilurin 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 at least one antifungal

compound from the family of strobilurin fungicides is selected from the group consisting of azoxystrobin, bifujunzhi, coumoxystrobin, dimoxystrobin, enestroburin, fluoxastrobin, jiexiangjunzhi, kresoxim-methyl, lvdngjunzhi, metominostrobin, oryastrobin, picoxystrobin, pyraclostrobin, pyrametostrobin, pyraoxystrobin, trifloxystrobin, and xiwojunan. In a preferred embodiment the at least one antifungal compound from the family of strobilurin fungicides is selected from the group consisting of azoxystrobin, dimoxystrobin, fluoxastrobin, kresoxim-methyl, metominostrobin, oryastrobin, picoxystrobin, pyraclostrobin and trifloxystrobin. In an even more preferred embodiment the at least one antifungal compound from the family of strobilurin fungicides selected from the group consisting of azoxystrobin, fluoxastrobin, kresoxim-methyl, picoxystrobin, pyraclostrobin and trifloxystrobin. 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.

In a specific embodiment the polyene antifungal compound and at least one antifungal compound from the family of strobilurin 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 strobilurin fungicides can for instance be used in the form of a pharmaceutical composition. The composition or compounds 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.

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 strobilurin fungicides. In an embodiment the at least one antifungal compound from the family of strobilurin fungicides is selected from the group consisting of azoxystrobin, bifujunzhi, coumoxystrobin, dimoxystrobin, enestroburin, fluoxastrobin, jiexiangjunzhi, kresoxim-methyl, lvdngjunzhi, metominostrobin, oryastrobin, picoxystrobin, pyraclostrobin, pyrametostrobin, pyraoxystrobin, trifloxystrobin, and xiwojunan. In a preferred embodiment the at least one antifungal compound from the family of strobilurin fungicides is selected from the group consisting of azoxystrobin, dimoxystrobin, fluoxastrobin, kresoxim-methyl, metominostrobin, oryastrobin, picoxystrobin, pyraclostrobin and trifloxystrobin. In an even more preferred embodiment the at least one antifungal compound from the family of strobilurin fungicides selected from the

group consisting of azoxystrobin, fluoxastrobin, kresoxim-methyl, picoxystrobin, pyraclostrobin and trifloxystrobin. 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 strobilurin fungicides. The treated products may comprise a polyene antifungal compound and at least one antifungal compound from the family of strobilurin 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/dm², preferably 0.00001 to 100 mg/dm², more preferably from 0.00005 to 10 mg/dm² of the polyene antifungal compound on their surface. In a further embodiment they comprise from 0.000001 to 200 mg/dm², preferably 0.00001 to 100 mg/dm², more preferably from 0.00005 to 10 mg/dm² of the at least one antifungal compound from the family of strobilurin 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.

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.

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.

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.

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.

5 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,
10 soy beans; oleaginous plants, *e.g.* rape, mustard, poppy, olive, sunflower, coconut, castor-oil plant, cocoa, ground-nuts; 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.*
15 spinach, lettuce, asparagus, brassicaceae such as cabbages and turnips, carrots, onions, tomatoes, potatoes, seed-potatoes, 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,
20 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.

 A method for preparing a composition as described herein is another aspect of
25 the present invention. The method comprises adding a polyene antifungal compound to at least one antifungal compound from the family of strobilurin fungicides. In an embodiment the at least one antifungal compound from the family of strobilurin fungicides is selected from the group consisting of azoxystrobin, bifujunzhi, coumoxystrobin, dimoxystrobin, enestroburin, fluoxastrobin, jiaxiangjunzhi, kresoxim-
30 methyl, lvdingjunzhi, metominostrobin, orysastrobin, picoxystrobin, pyraclostrobin, pyrametostrobin, pyraoxystrobin, trifloxystrobin, and xiwojunan. In a preferred embodiment the at least one antifungal compound from the family of strobilurin fungicides is selected from the group consisting of azoxystrobin, dimoxystrobin, fluoxastrobin, kresoxim-methyl, metominostrobin, orysastrobin, picoxystrobin,

pyraclostrobin and trifloxystrobin. In an even more preferred embodiment the at least one antifungal compound from the family of strobilurin fungicides selected from the group consisting of azoxystrobin, fluoxastrobin, kresoxim-methyl, picoxystrobin, pyraclostrobin and trifloxystrobin. 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

Pre-harvest application

Leaves of banana plants are inoculated with fungi. As a control non-inoculated leaves are also included. Next, a defined part of the leaves are treated with composition 1 (natamycin), composition 2 (azoxystrobin), composition 3 (kresoxim-methyl), composition 4 (metominostrobin), composition 5 (trifloxystrobin), composition 6 (picoxystrobin), composition 7 (pyraclostrobin), composition 8 (dimoxystrobin), composition 9 (fluoxastrobin), composition 10 (orysastrobin), composition 11 (natamycin + azoxystrobin), composition 12 (natamycin + kresoxim-methyl), composition 13 (natamycin + metominostrobin), composition 14 (natamycin + trifloxystrobin), composition 15 (natamycin + picoxystrobin), composition 16 (natamycin + pyraclostrobin), composition 17 (natamycin + dimoxystrobin), composition 18 (natamycin + fluoxastrobin) or composition 19 (natamycin + orysastrobin). Each composition is applied by spraying. Untreated leaves are also included (untreated control).

The obtained results show that the compositions of the present invention protect banana plants from fungal growth and further demonstrate that the compositions of the present invention show a synergistically enhanced activity compared to the activity of the active compounds when applied individually.

Example 2

Post-harvest application

Bananas are injured according to the method described by de Lapeyre de Bellaire and Dubois (1987). Bananas are wounded using a cork borer followed by contamination with fungal spores. After incubation for several hours at room temperature, the bananas are dipped in one of the following compositions: a) no treatment (control 1), b) dipped in water (control 2), c) dipped in natamycin, d) dipped in azoxystrobin, e) dipped in kresoxim-methyl, f) dipped in metominostrobin, g) dipped in trifloxystrobin, h) dipped in picoxystrobin, i) dipped in pyraclostrobin, j) dipped in dimoxystrobin, k) dipped in fluoxastrobin, l) dipped in oryastrobin, m) dipped in natamycin + azoxystrobin, n) dipped in natamycin + kresoxim-methyl, o) dipped in natamycin + metominostrobin, p) dipped in natamycin + trifloxystrobin, q) dipped in natamycin + picoxystrobin, r) dipped in natamycin + pyraclostrobin, s) dipped in natamycin + dimoxystrobin, t) dipped in natamycin + fluoxastrobin, u) dipped in natamycin + oryastrobin. After this treatment the bananas are incubated in closed boxes at 21°C at elevated humidity. Each day the bananas are judged visually on fungal development.

The results show that the composition comprising natamycin and at least one antifungal compound from the family of strobilurin fungicides protects bananas better against fungi than natamycin or at least one antifungal compound from the family of strobilurin fungicides alone. Surprisingly, the combined application of natamycin and at least one antifungal compound from the family of strobilurin fungicides leads to a strong synergistic reduction in infection.

Example 3

Treatment of bananas

Four organic, unripe (green) bananas were used per treatment. The peel of each banana was wounded thrice using a cork borer according to the method described by de Lapeyre de Bellaire and Dubois (1987). Subsequently, each wound was 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 was treated with 100 µl of a freshly prepared aqueous antifungal composition comprising either 500 ppm natamycin (DSM Food Specialties, Delft, The Netherlands), 1000 ppm fluoxastrobin or both. In addition, the antifungal compositions comprised 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

compositions was 4. A composition without natamycin or fluoxastrobin was used as control. The treated, unripe bananas were incubated in a closed box in the dark at 20°C and a relative air humidity of 95%, which was obtained in the presence of a saturated Na₂HPO₄ aqueous solution. During the first 10 days of incubation, a ripe (yellow) banana was included in the closed box to elevate the ethylene gas level and thus induce ripening of the treated, unripe bananas.

During incubation, the degree of mould growth on the bananas was assessed in a twofold manner: (i) the number of moulded wounds per total of 12 wounds was counted; and (ii) the antifungal activity (in %) of the individual active ingredients was 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 was calculated according to the Colby equation (Colby, 1967):

15

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

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.

The results in Table 1 (number of moulded wounds per total of 12 wounds) and Table 2 (antifungal activity) clearly demonstrate that the antifungal composition comprising both 500 ppm natamycin and 1000 ppm fluoxastrobin protected bananas better against mould growth than natamycin or fluoxastrobin alone.

After 21 days of incubation, all 12 wounds treated with the control composition showed mould growth, whereas 6 of the 12 wounds treated with natamycin and 10 of the 12 treated with fluoxastrobin were moulded. However, mould growth was observed for only 1 of the 12 wounds treated with the composition comprising both natamycin and fluoxastrobin (see Table 1). Furthermore, the observed antifungal activity of the composition comprising both natamycin and fluoxastrobin was 8% higher than the expected antifungal activity and a synergy factor of > 1.0 was obtained (see Table 2).

After 27 days of incubation, all 12 wounds treated with either the control composition, natamycin alone or fluoxastrobin alone showed mould growth. However, mould growth was observed only for 2 of the 12 wounds treated with the composition comprising both natamycin and fluoxastrobin (see Table 1). Moreover, the observed antifungal activity of the composition comprising both natamycin and fluoxastrobin was more than 30% higher than the expected antifungal activity and a synergy factor of 1.5 was obtained (see Table 2).

Hence, the combination of 500 ppm natamycin and 1000 ppm fluoxastrobin has synergistic antifungal activity on bananas.

Example 4

Treatment of bananas

The experiment was conducted as described in Example 3, except for the fact that each inoculated banana wound was treated with 100 µl of a freshly prepared aqueous antifungal composition comprising either 250 ppm natamycin (DSM Food Specialties, Delft, The Netherlands), 500 ppm fluoxastrobin or both. The antifungal activity (in %) of the individual and combined active ingredients on the banana wounds was determined according to the method described in Example 3.

The results in Table 3 (antifungal activity) reveal that the antifungal composition comprising 250 ppm natamycin as well as 500 ppm fluoxastrobin was superior to the compositions comprising either natamycin or fluoxastrobin in reducing mould growth on bananas.

After 27 days of incubation, the observed antifungal activity of the composition comprising both natamycin and fluoxastrobin was 8% higher than the expected antifungal activity. Consequently, the synergy factor exceeded 1.0 (see Table 3).

In conclusion, the results of this example clearly demonstrate that the antifungal activity of the combination of 250 ppm natamycin and 500 ppm fluoxastrobin is synergistic when applied on bananas.

Example 5*Treatment of bananas*

The experiment was conducted as described in Example 3, except for the fact that each inoculated banana wound was treated with 100 µl of a freshly prepared aqueous antifungal composition comprising either 500 ppm natamycin (DSM Food Specialties, Delft, The Netherlands), 1000 ppm trifloxystrobin or both. A composition without natamycin or trifloxystrobin was used as control. The degree of mould growth on the banana wounds was assessed according to the two methods described in Example 3.

The results in Table 4 (number of moulded wounds per total of 12 wounds) and Table 5 (antifungal activity) show that the combined antifungal composition comprising 500 ppm natamycin and 1000 ppm trifloxystrobin protected bananas more effectively against mould growth on bananas than the compositions comprising natamycin or trifloxystrobin alone.

After 27 days incubation, all 12 wounds treated with either the control composition or natamycin showed mould growth, whereas 10 of the 12 wounds treated with trifloxystrobin were moulded. However, when treated with the composition comprising both natamycin and trifloxystrobin, none of the 12 wounds were moulded (see Table 4). In addition, the actually observed antifungal activity of the composition comprising both natamycin and trifloxystrobin was nearly 10% higher than the expected antifungal activity, which resulted in a synergy factor > 1.0 (see Table 5).

Thus, the combined application of 500 ppm natamycin and 1000 ppm trifloxystrobin leads to a synergistic reduction in mould growth on bananas.

Example 6*Treatment of strawberries*

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 2-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), 1000 ppm trifloxystrobin or both. The antifungal composition also comprised 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 compositions was 4. A composition without natamycin or trifloxystrobin was used as control.

5 The treated strawberries were incubated in a closed box in the dark at 20°C and assessed on mould growth after 4, 5, 7 and 8 days of incubation. The degree of 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 active ingredients was determined by calculating the reduction in mould growth observed on the strawberries treated with the antifungal
10 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):

$$15 \quad E = X + Y - [(X \cdot Y) / 100]$$

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
20 synergy factor O/E is thus > 1.0, the combined application of the active ingredients leads to a synergistic antifungal effect.

The results in Table 6 (number of moulded strawberries per total of 12 strawberries) and Table 7 (antifungal activity) clearly demonstrate that the antifungal composition comprising 500 ppm natamycin and 1000 ppm trifloxystrobin had a much
25 stronger antifungal activity on strawberries than the compositions comprising natamycin or trifloxystrobin alone.

After 4 days of incubation, all 12 strawberries treated with either the control composition or trifloxystrobin showed mould growth, whereas 3 of the 12 strawberries treated with natamycin were moulded. However, mould growth was observed only for 1
30 of the 12 strawberries treated with the composition comprising natamycin and trifloxystrobin (see Table 6)

After 5 days of incubation, all 12 strawberries treated with either the control composition, natamycin or trifloxystrobin were moulded. However, mould growth was observed for only 8 of the 12 strawberries treated with the composition comprising

natamycin and trifloxystrobin. In addition, after 5 days of incubation the observed antifungal activity of the combined composition comprising natamycin and trifloxystrobin was > 30% higher than the expected antifungal activity, which resulted in a synergy factor of 2.0.

5 After 7 and 8 days of incubation, the observed antifungal activity of the composition comprising both natamycin and trifloxystrobin was respectively 15 and 18% higher than the expected antifungal activity. Consequently, the corresponding synergy factors exceeded 1.0 by far.

10 Hence, the combined application of 500 ppm natamycin and 1000 ppm trifloxystrobin synergistically reduces mould growth on strawberries.

Example 7

Treatment of strawberries

15 The experiment was conducted as described in Example 6, 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), 500 ppm trifloxystrobin or both. The treated strawberries were assessed on mould growth after 5, 7, 8 and 9 days of incubation according to the two methods described in Example 6.

20 The results in Table 8 (number of moulded strawberries per total of 12 strawberries) and Table 9 (antifungal activity) reveal that the antifungal composition comprising 250 ppm natamycin as well as 500 ppm trifloxystrobin was superior to the compositions comprising either natamycin or trifloxystrobin in reducing mould growth on bananas.

25 After 5 days of incubation, all 12 strawberries treated with either the control composition, natamycin or trifloxystrobin were moulded. However, mould growth was observed for only 9 of the 12 strawberries treated with the composition comprising natamycin and trifloxystrobin (see Table 8).

30 After 7, 8 and 9 days of incubation, the observed antifungal activity of the composition comprising natamycin and trifloxystrobin was 17 to 27% higher than the expected antifungal activity. The corresponding synergy factor exceeded 1.0 on each of these three consecutive days and even increased from 3.7 on day 7 to >17 on day 9 (see Table 9).

Thus, the combined application of 250 ppm natamycin and 500 ppm trifloxystrobin results in a surprisingly strong synergistic reduction in mould growth on strawberries.

5 **Example 8**

Treatment of strawberries

The experiment was conducted as described in Example 6, 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 50 ppm natamycin
10 (DSM Food Specialties, Delft, The Netherlands), 250 ppm trifloxystrobin or both. After 3, 4, and 5 days of incubation, the antifungal activity (in %) of the individual and combined active ingredients on the treated strawberries was determined according to the method described in Example 6.

The results (see Table 10) show that the combined antifungal composition
15 comprising 50 ppm natamycin and 250 ppm trifloxystrobin protected strawberries better against mould growth than the compositions comprising natamycin alone or trifloxystrobin alone.

After 3, 4, and 5 days of incubation, the observed antifungal activity of composition comprising natamycin and trifloxystrobin exceeded the expected antifungal
20 activity with 9 to 18% and synergy factors > 1.0 were obtained.

In conclusion, the antifungal activity of the active ingredient combination of 50 ppm natamycin and 250 ppm trifloxystrobin is synergistic when applied on strawberries.

Example 9

25 *Treatment of mandarins*

Ten fresh, organic mandarins were used per treatment. The peel of each mandarin 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 1×10^4 of spores/ml. After
30 incubation for 2 hours at 20°C, the mandarins were dipped individually for 1 minute in a freshly prepared aqueous antifungal composition comprising either 250 ppm natamycin (DSM Food Specialties, Delft, The Netherlands), 500 ppm fluoxastrobin or both. In addition, the antifungal composition comprised 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 compositions was 4. A composition without natamycin or fluoxastrobin was used as control.

5 The treated mandarins were incubated in a closed box in the dark at 20°C and assessed on mould growth after 10, 15, 19, 22 and 27 days of incubation. The antifungal activity (in %) of the individual active ingredients was 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
10 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):

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

15

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
20 to a synergistic antifungal effect.

The results (see Table 11) clearly demonstrate that the antifungal composition comprising 250 ppm natamycin and 500 ppm fluoxastrobin was superior to the compositions comprising natamycin or fluoxastrobin alone in preventing mould growth on mandarins.

25

After 10, 15, 19, 22 and 27 days of incubation, the observed antifungal activity of the composition comprising both natamycin and fluoxastrobin was 9 to 20% higher than the expected antifungal activity. Consequently, the obtained synergy factors were >1.0 and ranged from 1.3 to 2.8.

30

Hence, the results of this example clearly demonstrate the synergistic antifungal activity between 250 ppm natamycin and 500 ppm fluoxastrobin on mandarins.

Example 10*Treatment of mandarins*

The experiment was conducted as described in Example 9, except for the fact that each wounded, inoculated mandarin was dipped individually for 1 minute in a freshly prepared aqueous antifungal composition comprising either 250 ppm natamycin (DSM Food Specialties, Delft, The Netherlands), 400 ppm trifloxystrobin or both. A composition without natamycin or trifloxystrobin was used as control. The antifungal activity (in %) of the individual and combined active ingredients on mandarins was assessed after 8, 10 and 19 days of incubation according to the method described in Example 9.

The results (see Table 12) prove that the antifungal composition comprising 250 ppm natamycin and 400 ppm trifloxystrobin was more effective in reducing mould growth on mandarins than natamycin or trifloxystrobin alone.

After 8, 10 and 19 days of incubation, the observed antifungal activity of the active ingredient combination of natamycin and trifloxystrobin exceeded the expected antifungal activity with 7 to 9%, which resulted in synergy factors > 1.0.

It can therefore be concluded, that the combined application of 250 ppm natamycin and 400 ppm trifloxystrobin leads to a synergistic reduction in mould growth on mandarins.

Table 1. Number of moulded wounds on bananas incubated at 20°C after treatment with compositions comprising either 500 ppm natamycin, 1000 ppm fluoxastrobin or both.

Antifungal composition	Incubation time (days)	Number of moulded wounds / total number of 12 wounds
Control	21	12/12
Natamycin 500 ppm		6/12
Fluoxastrobin 1000 ppm		10/12
Natamycin 500 ppm + fluoxastrobin 1000 ppm		1/12
Control	27	12/12
Natamycin 500 ppm		12/12
Fluoxastrobin 1000 ppm		12/12
Natamycin 500 ppm + fluoxastrobin 1000 ppm		2/12

Table 2. Antifungal activity (%) of compositions comprising either 500 ppm natamycin, 1000 ppm fluoxastrobin or both on bananas after incubation at 20°C.

Antifungal composition	Incubation time (days)	Observed antifungal activity O (%)	Expected antifungal activity E (%)	Synergy factor O/E
Control	21	0	-	-
Natamycin 500 ppm		78	-	-
Fluoxastrobin 1000 ppm		42	-	-
Natamycin 500 ppm + fluoxastrobin 1000 ppm		95	87	1.1
Control	27	0	-	-
Natamycin 500 ppm		63	-	-
Fluoxastrobin 1000 ppm		7	-	-
Natamycin 500 ppm + fluoxastrobin 1000 ppm		97	66	1.5

5 Table 3. Antifungal activity (%) of compositions comprising either 250 ppm natamycin, 500 ppm fluoxastrobin or both on bananas after incubation at 20°C.

Antifungal composition	Incubation time (days)	Observed antifungal activity O (%)	Expected antifungal activity E (%)	Synergy factor O/E
Control	27	0	-	-
Natamycin 250 ppm		4	-	-
Fluoxastrobin 500 ppm		31	-	-
Natamycin 250 ppm + fluoxastrobin 500 ppm		42	34	1.2

Table 4. Number of moulded wounds on bananas incubated at 20°C after treatment with compositions comprising either 500 ppm natamycin, 1000 ppm trifloxystrobin or both.

Antifungal composition	Incubation time (days)	Number of moulded wounds / total number of 12 wounds
Control	27	12/12
Natamycin 500 ppm		12/12

Trifloxystrobin 1000 ppm		10/12
Natamycin 500 ppm + trifloxystrobin 1000 ppm		0/12

Table 5. Antifungal activity (%) of compositions comprising either 500 ppm natamycin, 1000 ppm trifloxystrobin or both on bananas after incubation at 20°C.

Antifungal composition	Incubation time (days)	Observed antifungal activity O (%)	Expected antifungal activity E (%)	Synergy factor O/E
Control	27	0	-	-
Natamycin 500 ppm		63	-	-
Trifloxystrobin 1000 ppm		75	-	-
Natamycin 500 ppm + trifloxystrobin 1000 ppm		100	91	1.1

- 5 Table 6. Number of moulded strawberries incubated at 20°C after treatment with compositions comprising either 500 ppm natamycin, 1000 ppm trifloxystrobin or both.

Antifungal composition	Incubation time (days)	Number of moulded strawberries / total number of 12 strawberries
Control	4	12/12
Natamycin 500 ppm		3/12
Trifloxystrobin 1000 ppm		12/12
Natamycin 500 ppm + Trifloxystrobin 1000 ppm		1/12
Control	5	12/12
Natamycin 500 ppm		12/12
Trifloxystrobin 1000 ppm		12/12
Natamycin 500 ppm + Trifloxystrobin 1000 ppm		8/12

Table 7. Antifungal activity (%) of compositions comprising either 500 ppm natamycin, 1000 ppm trifloxystrobin 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	5	0	-	-
Natamycin 500 ppm		31	-	-
Trifloxystrobin 1000 ppm		0	-	-
Natamycin 500 ppm + trifloxystrobin 1000 ppm		63	31	2.0
Control	7	0	-	-
Natamycin 500 ppm		13	-	-
Trifloxystrobin 1000 ppm		6	-	-
Natamycin 500 ppm + trifloxystrobin 1000 ppm		33	18	1.8
Control	8	0	-	-
Natamycin 500 ppm		0	-	-
Trifloxystrobin 1000 ppm		6	-	-
Natamycin 500 ppm + trifloxystrobin 1000 ppm		24	6	4.0

5 Table 8. Number of moulded strawberries incubated at 20°C after treatment with compositions comprising either 250 ppm natamycin, 500 ppm trifloxystrobin or both.

Antifungal composition	Incubation time (days)	Number of moulded strawberries / total number of 12 strawberries
Control	5	12/12
Natamycin 250 ppm		12/12
Trifloxystrobin 500 ppm		12/12
Natamycin 250 ppm + Trifloxystrobin 500 ppm		9/12

Table 9. Antifungal activity (%) of compositions comprising either 250 ppm natamycin, 500 ppm trifloxystrobin 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	7	0	-	-
Natamycin 250 ppm		10	-	-
Trifloxystrobin 500 ppm		0	-	-
Natamycin 250 ppm + trifloxystrobin 500 ppm		37	10	3.7
Control	8	0	-	-
Natamycin 250 ppm		2	-	-
Trifloxystrobin 500 ppm		0	-	-
Natamycin 250 ppm + trifloxystrobin 500 ppm		24	2	12
Control	9	0	-	-
Natamycin 250 ppm		0	-	-
Trifloxystrobin 500 ppm		0	-	-
Natamycin 250 ppm + trifloxystrobin 500 ppm		17	0	>17

Table 10. Antifungal activity (%) of compositions comprising either 50 ppm natamycin, 250 ppm trifloxystrobin 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	3	0	-	-
Natamycin 50 ppm		64	-	-
Trifloxystrobin 250 ppm		50	-	-
Natamycin 50 ppm + trifloxystrobin 250 ppm		100	82	1.2
Control	4	0	-	-
Natamycin 50 ppm		50	-	-
Trifloxystrobin 250 ppm		44	-	-
Natamycin 50 ppm		81	72	1.1

+ trifloxystrobin 250 ppm				
Control	5	0	-	-
Natamycin 50 ppm		17	-	-
Trifloxystrobin 250 ppm		7	-	-
Natamycin 50 ppm + trifloxystrobin 250 ppm		43	23	1.9

Table 11. Antifungal activity (%) of compositions comprising either 250 ppm natamycin, 500 ppm fluoxastrobin or both on mandarins after incubation at 20°C.

Antifungal composition	Incubation time (days)	Observed antifungal activity O (%)	Expected antifungal activity E (%)	Synergy factor O/E
Control	10	0	-	-
Natamycin 250 ppm		54	-	-
Fluoxastrobin 500 ppm		28	-	-
Natamycin 250 ppm + fluoxastrobin 500 ppm		87	67	1.3
Control	15	0	-	-
Natamycin 250 ppm		19	-	-
Fluoxastrobin 500 ppm		24	-	-
Natamycin 250 ppm + fluoxastrobin 500 ppm		48	38	1.3
Control	19	0	-	-
Natamycin 250 ppm		5	-	-
Fluoxastrobin 500 ppm		6	-	-
Natamycin 250 ppm + fluoxastrobin 500 ppm		24	11	2.2
Control	22	0	-	-
Natamycin 250 ppm		5	-	-
Fluoxastrobin 500 ppm		4	-	-
Natamycin 250 ppm + fluoxastrobin 500 ppm		20	9	2.2
Control	27	0	-	-

Natamycin 250 ppm		5	-	-
Fluoxastrobin 500 ppm		0	-	-
Natamycin 250 ppm + fluoxastrobin 500 ppm		14	5	2.8

Table 12. Antifungal activity (%) of compositions comprising either 250 ppm natamycin, 400 ppm trifloxystrobin or both on mandarins 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		83	-	-
Trifloxystrobin 400 ppm		61	-	-
Natamycin 250 ppm + trifloxystrobin 400 ppm		100	93	1.1
Control	10	0	-	-
Natamycin 250 ppm		54	-	-
Trifloxystrobin 400 ppm		42	-	-
Natamycin 250 ppm + trifloxystrobin 400 ppm		82	73	1.1
Control	19	0	-	-
Natamycin 250 ppm		5	-	-
Trifloxystrobin 400 ppm		0	-	-
Natamycin 250 ppm + trifloxystrobin 400 ppm		13	5	2.6

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CLAIMS

1. A composition comprising a polyene antifungal compound and trifloxystrobin.
- 5 2. A composition according to claim 1, wherein the polyene antifungal compound is natamycin.
3. A composition according to claim 1 or 2, wherein the composition further comprises
10 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
15 UV-absorber and an antioxidant.
4. A composition according to any one of the claims 1 to 3, 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 trifloxystrobin is in the range from about 0.0001 g/l to about 2000 g/l.
20
5. A kit comprising a polyene antifungal compound and trifloxystrobin.
6. A method for protecting an agricultural product against fungi by treating the product with a polyene antifungal compound and at least one antifungal compound from the
25 family of strobilurin fungicides.
7. A method according to claim 6, wherein the at least one antifungal compound from the family of strobilurin fungicides is selected from the group consisting of azoxystrobin, bifujunzhi, coumoxystrobin, dimoxystrobin, enestroburin, fluoxastrobin, jiaxiangjunzhi, kresoxim-methyl, lvdingjunzhi, metominostrobin, orysastrobin,
30 picoxystrobin, pyraclostrobin, pyrametostrobin, pyraoxystrobin, trifloxystrobin, and xiwojunan.
8. A method according to claim 6 or 7, wherein the product is treated post-harvest.

9. An agricultural product comprising a polyene antifungal compound and at least one antifungal compound from the family of strobilurin fungicides.
- 5 10. Use of a polyene antifungal compound and at least one antifungal compound from the family of strobilurin fungicides to protect an agricultural product against fungi.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/053507

A. CLASSIFICATION OF SUBJECT MATTER					
INV.	A01N43/90	A01N43/54	A01N37/50	A01N43/88	A01N43/40
	A01N47/24	A01N43/56	A01N43/16	A01P1/00	A01P3/00
	A23B7/14				
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 A23B

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 practical, 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.
Y	WO 02/00027 A1 (MARTINI ALFREDO [IT]; GOBBI PIERGIORGIO [IT]) 3 January 2002 (2002-01-03) page 1, line 3 - line 9 page 4, line 15 - line 27 page 6, line 4 - line 12 example 6 claim 11 ----- -/--	1,3-10

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

<p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>
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Date of the actual completion of the international search 25 April 2012	Date of mailing of the international search report 07/05/2012
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Zanobini, Alessandra
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/053507

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>WO 2007/104677 A1 (BASF AG [DE]; KOEHLE HARALD [DE]; BARDINELLI TED R [US]; TAVARES-RODRI)</p> <p>20 September 2007 (2007-09-20)</p> <p>page 2, line 11 - line 14</p> <p>page 10, line 2 - line 6</p> <p>page 11, line 10 - line 13</p> <p>page 12, line 1 - line 13</p> <p>page 13, line 21 - line 33</p> <p>page 15, line 1 - page 16, line 22</p> <p>-----</p>	1,3-10
A	<p>WO 2007/104669 A2 (BASF AG [DE]; KOEHLE HARALD [DE]; BARDINELLI TED R [US]; TAVARES-RODRI)</p> <p>20 September 2007 (2007-09-20)</p> <p>page 1, line 5 - page 3, line 16</p> <p>page 11, line 7 - line 10</p> <p>page 13, line 19 - line 31</p> <p>page 14, line 36 - page 15, line 3</p> <p>page 18, line 23 - page 20, line 3</p> <p>-----</p>	1-10
A	<p>WO 2009/077613 A1 (DSM IP ASSETS BV [NL]; PLANT RES INT BV [NL]; STARK JACOBUS [NL]; VAN) 25 June 2009 (2009-06-25)</p> <p>page 1, line 5 - line 11</p> <p>page 5, line 1 - line 23</p> <p>table 3</p> <p>-----</p>	1-10

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International application No

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