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(54) Title: PLANT HEALTH EFFECT OF PURPUREOCILLIUM LILACINUM

(57) Abstract: The present invention relates to a method for promoting or improving plant health and/or plant growth of agricultural plants wherein the plants, the plant propagules, the seed of the plants and/or the locus where the plants are growing or are intended to grow are treated with an effective amount of a composition comprising the fungus *Purpureocillium lilacinum* or spores thereof. Further aspects of the invention relate to uses of a composition comprising the fungus *Purpureocillium lilacinum* or spores thereof for promoting or improving plant health and/or plant growth.



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**Plant Health Effect of *Purpureocillium Lilacinum***

The use of plant protection formulations comprising biological control agents (BCAs) has become a valuable alternative in the field of plant protection. Biological control agents directed against fungi or insects as well as those promoting plant health have been put on the market in different formulations.

5

A number of *Purpureocillium lilacinum* (formerly known as *Paecilomyces lilacinus*) strains have been described for use as a biological control agent. Such strains include strain 251 in the products BIOACT<sup>®</sup>, MELOCON<sup>®</sup> and NEMOUT<sup>®</sup> produced by Bayer CropScience Biologics GmbH, a strain 580 in the product BIOSTAT<sup>®</sup> WP (ATCC No. 38740) produced by Laverlam, a strain in the product BIO-NEMATON<sup>®</sup> produced by the company T.Stanes and Company Ltd., a strain in the product MYSIS<sup>®</sup> produced by the company Varsha Bioscience and Technology India Pvt Ltd., one in the product BIOICONEMA<sup>®</sup> available from Nico Orgo Maures, India, one in the product NEMAT<sup>®</sup>, available from Ballagro Agro Tecnologia Ltda, Brazil and one in the product SPECTRUM PAE L<sup>®</sup> available from Promotora Tecnica Industrial, S.A. DE C.V., Mexico. Those strains of the species are known to have a nematicidal effect.

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A plant health or plant growth promoting effect has been reported for several biological control agents such as e.g., *Penicillium bilaii* which enhances phosphorous uptake efficiency. For *P. lilacinum*, no such plant growth promoting (PGP) or plant health effect has been reported so far.

20 Accordingly, in a first aspect, the present invention relates to a method for promoting or improving plant health and/or plant growth of agricultural plants wherein the plants, the plant propagules, the seed of the plants and/or the locus where the plants are growing or are intended to grow are treated with an effective amount of a composition comprising the fungus *Purpureocillium lilacinum* or spores thereof.

25 As mentioned above, several *P. lilacinum* strains are known. Such strains include strain 251 in the products BIOACT<sup>®</sup>, MELOCON<sup>®</sup> and NEMOUT<sup>®</sup> produced by Bayer CropScience Biologics GmbH, a strain 580 in the product BIOSTAT<sup>®</sup> WP (ATCC no. 38740) produced by Laverlam, a strain in the product BIO-NEMATON<sup>®</sup> produced by the company T.Stanes and Company Ltd., a strain in the product MYSIS<sup>®</sup> produced by the company Varsha Bioscience and Technology India Pvt Ltd., one in the product  
30 BIOICONEMA<sup>®</sup> available from Nico Orgo Maures, India, one in the product NEMAT<sup>®</sup>, available from Ballagro Agro Tecnologia Ltda, Brazil and one in the product SPECTRUM PAE L<sup>®</sup> available from Promotora Tecnica Industrial, S.A. DE C.V., Mexico. Those strains of the species are known to have a nematicidal effect.

In a preferred embodiment, promoting or improving plant health comprises achieving and/or manifests in improved stress tolerance, less dead basal leaves, greener leaf color, higher pigment content, improved photosynthetic activity and enhanced plant vigor. All of these properties as well as the one listed further below are measured in comparison with plants which were not treated with *P. lilacinum* but were otherwise  
5 grown under the same conditions

In another preferred embodiment, promoting or improving plant growth comprises or manifests in tillering increase, increase in plant height, bigger leaf blade, bigger leaf surface, stronger tillers, timing of, e.g. earlier, flowering, reduced blossom drop, early grain maturity, earlier or prolonged fruit set, less plant verse  
10 (lodging), increased shoot growth, increased plant stand, early and/or better germination, earlier and/or increased emergence, improved crop yield, improved total vegetative weight or whole plant biomass, improved protein content, improved oil content, improved starch content, improved root growth (including root length), improved root size (including root surface), improved root weight and/or improved root effectiveness, improved shoot weight, increased root weight, increased plant biomass and improved fruit  
15 weight. The skilled person is aware that in some cases, the scope of terms here listed as falling within plant health may as well extend to plant growth and vice versa.

In another more preferred embodiment, improved stress tolerance comprises improved tolerance to drought, heat, salt, UV, water cold and/or xenobiotic conditions.  
20

In a more preferred embodiment, plant growth refers to leaf surface, root growth, root size, root weight, fruit weight, shoot weight, plant biomass and/or crop yield.

Any characteristic listed above for promoting or improving plant health and/or plant growth may be  
25 improved by at least 3%, preferably at least 5%, more preferably at least 10%, at least 15% or even at least 20% or at least 25% or in some instances even more than 30% or 35% as compared to that of plants not treated with said composition or treated with a blank formulation. Most preferably, for this and the following embodiments, plants are otherwise treated in the same manner.

30 For example, the composition according to the invention may result in an increased shoot weight of at least 3%, preferably at least 5%, more preferably at least 10%, at least 13%, at least 15% or at least 20% as compared to that of plants not treated with said composition or treated with a formulation not comprising *P. lilacinum* (in the examples also referred to as "blank"). In some cases shoot weight may even be increased by at least 25% or more.  
35

An increase in plant biomass in plants treated with the composition according to the invention is preferably at least 3%, preferably at least 5%, more preferably at least 10%, at least 15% or even at least 20% or at least 25% or at least 30% or even more as compared to that of plants not treated with said composition or treated with a blank formulation.

5

Root weight of plants treated according to the invention may be increased by at least 3%, preferably at least 5%, more preferably at least 10%, at least 15%, at least 20% or even at least 25% as compared to that of plants not treated with said composition or treated with a blank formulation. Similarly, treatment according to the invention may increase the root surface of a plant by at least 3%, at least 5%, at least 10% or at least 15%. In this way, root length of plants may be increased by 3%, preferably at least 5%, more preferably at least 10%, at least 20% or even at least 25% as compared to that of plants not treated with said composition or treated with a blank formulation.

An increase in leaf surface area biomass in plants treated with the composition according to the invention may be at least 3%, preferably at least 5%, more preferably at least 8%, at least 10%, at least 14% or even at least 20% or at least 22% as compared to that of plants not treated with said composition or treated with a blank formulation.

Fruit weight of plants treated according to the invention may be increased by at least 3%, preferably at least 5%, more preferably at least 10%, at least 15%, at least 20% or even at least 25% or at least 30% as compared to that of plants not treated with said composition or treated with a blank formulation.

As can be seen in the examples, application of a composition comprising *P. lilacinum* or spores thereof resulted in improved plant growth, in particular in improved root growth, root size, improved fruit weight, improved shoot weight and thus in improved crop yield, both under and most notably also with reduced or absent nematode pressure.

In the course of the present invention, it was surprisingly found that the improvement in root growth, root size, shoot growth, plant biomass, leaf surface area and crop yield (in the form of fruit weight) exceeds the expectations based on the plant protection, i.e., nematicidal effect, of *P. lilacinum*. This means that application of *P. lilacinum* upon infection with nematodes does not only have an effect to reduce the damage done by said nematodes but provides an additional plant health effect or plant growth promotion effect in the form of a disproportionately high yield and improved plant characteristics. This is demonstrated in example 1 appended to this application clearly showing that the nematode efficacy of *P. lilacinum* is not correlated with the plant health and plant growth effects observed upon treatment. It was further confirmed

by the different conditions used that said effects are not correlated with certain environmental factors such as organic matter (OM), pH, soil texture and soil temperature.

5 In a preferred embodiment said *Purpureocillium lilacinum* is strain 251 as described in WO 1991/002051 or a mutant thereof having all identifying characteristics of the respective strain. The strain 251 has been isolated from a Meloidogyne egg mass in Los Banos, Philippines, and has been deposited with the Australian Government Analytical Laboratories (AGAL) in 1989 under the Accession No. 89/030550. In this regard, the identifying characteristics of the strain relate to those defining the nematocidal activity and the PGP or plant health promoting activity. Accordingly, a mutant strain of *P. lilacinum* 251 still possesses  
10 the PGP/plant health effects and preferably also the nematocidal effects described further above but may differ in other properties such as e.g. storage stability of the spores produced by the fungus.

Exemplary commercial products containing *Purpureocillium lilacinum* strain 251 are BIOACT® WG and MELOCON® WG. Liquid formulations comprising spores of said strain 251 are disclosed in  
15 WO2012/163322 and WO2016/050726, both of which are incorporated herein by reference. The activity of *Purpureocillium lilacinum* strain 251 is described inter alia in A. Khan *et al.*, FEMS Microbiology Letters, 227, 107-111, 2003 and S. Kiewnick *et al.*, Biological Control 38, 179-187, 2006. Its isolation and characteristic properties are disclosed in WO 91/02051, which is incorporated herein by reference.

20 In another preferred embodiment, said promoting or improving plant health and/or plant growth is independent of pathogenic nematode pressure.

Whereas it was found that the PGP or plant health effect of *P. lilacinum* application occurs in plants infested with nematodes, it was also confirmed that this effect is actually independent of any nematode infestations.  
25 In example 1, it can be seen that the effect on yield is present whereas the nematocidal efficacy is sometimes not at its maximum. This confirms that the effects observed are not correlated with the nematocidal activity of the fungus.

In a more preferred embodiment, said promoting or improving plant health and/or plant growth is in the  
30 absence of pathogenic nematode pressure.

This effect on PGP or plant health is demonstrated in example 6 and shown on the exemplary crops tomato and cucumber.

*Purpureocillium lilacinum* is cultivated according to methods known in the art on an appropriate substrate, e.g., by submerged fermentation or solid-state fermentation, e.g., using a device disclosed in WO 2005/012478 or WO 1999/057239. Subsequently, the fungus or its organs, such as the spores or conidia is/are separated from the substrate. The substrate populated with the microorganism or the conidia is dried preferably before the separation step. After separation from the substrate, the microorganism or its organs may be dried via e.g., freeze-drying, vacuum drying or spray drying after separation.

10

The term spores normally includes sexually (e.g., oospores, zygospores or ascospores) and asexually (e.g., conidia and chlamydospores, but also uredospores, teleutospores and ustospores) formed spores.

The only kind of spores formed by *P. lilacinum* are conidia so that this form of spores is preferred herein.

15

In a more preferred embodiment said spores are dried spores. Formulations comprising dried spores have been shown to have a longer shelf-life so that such formulations are applicable for a longer time as compared to aqueous formulations or those comprising spores which have not been dried.

20

Methods for preparing dried spores are well known in the art and include fluidized bed drying, spray drying, vacuum drying and lyophilization. Conidia may be dried in 2 steps: For conidia produced by solid-state fermentation first the conidia covered culture substrate is dried before harvesting the conidia from the dried culture substrate thereby obtaining a pure conidia powder. Then the conidia powder is dried further using vacuum drying or lyophilization before formulating it according to the invention. In liquid formulations comprising *P. lilacinum* spores as described in WO2016/050726, preferably, the polyether-modified trisiloxane and fumed silica or precipitated silica are combined in the desired ratio according to methods well-known in the art and provided e.g., in manufacturer's instructions, to form a carrier according to the invention. For example, such method of preparing a carrier includes applying high shear to disperse the fumed silica or precipitated silica in the polyether-modified trisiloxane to result in a homogenous mixture prior to mixing with the biological control agent and optionally further ingredients in the desired ratio. Preferably the polyether-modified trisiloxane is circulated from a receiving vessel via a rotor/stator machine, and the silica powder is introduced, using a feed device, into the shear zone between the slots in the rotor teeth and the stator slots, continuously or discontinuously, and with the rotor stator machine running, the feed device closes and shearing continues in such a way that the shear rate is in the range of between 1000 and 10000 s<sup>-1</sup>.

30

In one preferred embodiment, seed is treated.

35

In other embodiments, treatment may be carried out in the form of any kind of soil application, such as in-furrow, by drip application, soil incorporation, drench application, sprinkler irrigation, micro injection or granule application.

5 The present composition may be applied to crops using any of the methods well known in the art. It may be advantageous to apply the inventive composition to the environment of the roots. This may be achieved by coating of the seeds with a composition comprising *P. lilacinum*, preferably spores (conidia) of *P. lilacinum*, so that emergence of roots results in a fungal inoculum in their environment; by dipping or spraying the root regions of seedlings or seed trays in a nursery situation, or by application of the composition at the site of  
10 planting, either in aqueous suspension or in solid form. It is particularly preferred that the inventive composition is specifically applied to the regions of the plant rhizosphere, preferably that affected by nematodes. Vegetables and other transplants can be treated just before transplanting e.g. with a soil drench.

It is preferred that application is done via drip irrigation.

15 In a preferred embodiment, said treatment is carried out in the soil, prior to germination of a seed and/or in the soil in contact with a root of said plant or where a plant is intended to grow.

For root development, it is most useful to apply the fungus prior to transplanting also due to its characteristic  
20 as egg parasite and onwards throughout cropping duration following nematode development. Alternatively or in addition, the fungus may be applied towards the late season, preferably after a treatment with a chemical plant protection agent.

In order to achieve beneficial yield effects, one or more applications of the fungus such as sequential  
25 applications, e.g., as shown in the examples may be carried out at any point prior to sowing/planting or during growth of the plant.

In some embodiments, the treatment is carried out once. In other embodiments, the treatment is carried out repeatedly.

30 In connection with this embodiment of the present invention, the term “repeatedly” refers to more than once. Accordingly, repeatedly may refer to at least two, at least three, at least four or even at least five applications of the fungus or spores thereof prior to sowing/planting and/or during germination and/or growth of the plant.

35

Usual application times can be derived from the appended examples as well as the instructions of commercially available products, however they can be derived based on the specific crop, pest pressure, kind of application and design by the skilled person.

- 5 In a preferred embodiment, the method of the invention further comprises applying, simultaneously or sequentially, at least one further plant protection agent.

Said plant protection agent may be a nematicide, an insecticide, a bactericide, a miticide, a fungicide or another agent promoting or improving plant health.

- 10 The following plant protection agents can, if their functional groups enable this, optionally form salts with suitable bases or acids.

Fungicides of the following classes (1) to (15) comprise:

- 15 1) Inhibitors of the ergosterol biosynthesis, for example, (1.001) cyproconazole, (1.002) difenoconazole, (1.003) epoxiconazole, (1.004) fenhexamid, (1.005) fenpropidin, (1.006) fenpropimorph, (1.007) fenpyrazamine, (1.008) fluquinconazole, (1.009) flutriafol, (1.010) imazalil, (1.011) imazalil sulfate, (1.012) ipconazole, (1.013) metconazole, (1.014) myclobutanil, (1.015) paclobutrazol, (1.016) prochloraz, (1.017) propiconazole, (1.019) Pyrisoxazole, (1.020) spiroxamine, (1.021) tebuconazole, (1.022) tetraconazole,  
 20 (1.023) triadimenol, (1.024) tridemorph, (1.025) triticonazole, (1.026) (1R,2S,5S)-5-(4-chlorobenzyl)-2-(chloromethyl)-2-methyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol, (1.027) (1S,2R,5R)-5-(4-chlorobenzyl)-2-(chloromethyl)-2-methyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol, (1.028) (2R)-2-(1-chlorocyclopropyl)-4-[(1R)-2,2-dichlorocyclopropyl]-1-(1H-1,2,4-triazol-1-yl)butan-2-ol, (1.029) (2R)-2-(1-chlorocyclopropyl)-4-[(1S)-2,2-dichlorocyclopropyl]-1-(1H-1,2,4-triazol-1-yl)butan-2-ol, (1.030) (2R)-2-  
 25 [4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol, (1.031) (2S)-2-(1-chlorocyclopropyl)-4-[(1R)-2,2-dichlorocyclopropyl]-1-(1H-1,2,4-triazol-1-yl)butan-2-ol, (1.032) (2S)-2-(1-chlorocyclopropyl)-4-[(1S)-2,2-dichlorocyclopropyl]-1-(1H-1,2,4-triazol-1-yl)butan-2-ol, (1.033) (2S)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol, (1.034) (R)-[3-(4-chloro-2-fluorophenyl)-5-(2,4-difluorophenyl)-1,2-oxazol-4-yl](pyridin-3-yl)methanol, (1.035) (S)-[3-(4-  
 30 chloro-2-fluorophenyl)-5-(2,4-difluorophenyl)-1,2-oxazol-4-yl](pyridin-3-yl)methanol, (1.036) [3-(4-chloro-2-fluorophenyl)-5-(2,4-difluorophenyl)-1,2-oxazol-4-yl](pyridin-3-yl)methanol, (1.037) 1-({(2R,4S)-2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl}methyl)-1H-1,2,4-triazole, (1.038) 1-  
 (1.039) 1-{{(2S,4S)-2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl}methyl)-1H-1,2,4-triazole,  
 35 thiocyanate, (1.040) 1-{{rel(2R,3R)-3-(2-chlorophenyl)-2-(2,4-difluorophenyl)oxiran-2-yl}methyl}-1H-

1,2,4-triazol-5-yl thiocyanate, (1.041) 1-{{rel(2R,3S)-3-(2-chlorophenyl)-2-(2,4-difluorophenyl)oxiran-2-yl}methyl}-1H-1,2,4-triazol-5-yl thiocyanate, (1.042) 2-[(2R,4R,5R)-1-(2,4-dichlorophenyl)-5-hydroxy-2,6,6-trimethylheptan-4-yl]-2,4-dihydro-3H-1,2,4-triazole-3-thione, (1.043) 2-[(2R,4R,5S)-1-(2,4-dichlorophenyl)-5-hydroxy-2,6,6-trimethylheptan-4-yl]-2,4-dihydro-3H-1,2,4-triazole-3-thione, (1.044) 2-[(2R,4S,5R)-1-(2,4-dichlorophenyl)-5-hydroxy-2,6,6-trimethylheptan-4-yl]-2,4-dihydro-3H-1,2,4-triazole-3-thione, (1.045) 2-[(2R,4S,5S)-1-(2,4-dichlorophenyl)-5-hydroxy-2,6,6-trimethylheptan-4-yl]-2,4-dihydro-3H-1,2,4-triazole-3-thione, (1.046) 2-[(2S,4R,5R)-1-(2,4-dichlorophenyl)-5-hydroxy-2,6,6-trimethylheptan-4-yl]-2,4-dihydro-3H-1,2,4-triazole-3-thione, (1.047) 2-[(2S,4R,5S)-1-(2,4-dichlorophenyl)-5-hydroxy-2,6,6-trimethylheptan-4-yl]-2,4-dihydro-3H-1,2,4-triazole-3-thione, (1.048) 2-[(2S,4S,5R)-1-(2,4-dichlorophenyl)-5-hydroxy-2,6,6-trimethylheptan-4-yl]-2,4-dihydro-3H-1,2,4-triazole-3-thione, (1.049) 2-[(2S,4S,5S)-1-(2,4-dichlorophenyl)-5-hydroxy-2,6,6-trimethylheptan-4-yl]-2,4-dihydro-3H-1,2,4-triazole-3-thione, (1.050) 2-[1-(2,4-dichlorophenyl)-5-hydroxy-2,6,6-trimethylheptan-4-yl]-2,4-dihydro-3H-1,2,4-triazole-3-thione, (1.051) 2-[2-chloro-4-(2,4-dichlorophenoxy)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol, (1.052) 2-[2-chloro-4-(4-chlorophenoxy)phenyl]-1-(1H-1,2,4-triazol-1-yl)butan-2-ol, (1.053) 2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)butan-2-ol, (1.054) 2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)pentan-2-ol, (1.055) 2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol, (1.056) 2-{{3-(2-chlorophenyl)-2-(2,4-difluorophenyl)oxiran-2-yl}methyl}-2,4-dihydro-3H-1,2,4-triazole-3-thione, (1.057) 2-{{rel(2R,3R)-3-(2-chlorophenyl)-2-(2,4-difluorophenyl)oxiran-2-yl}methyl}-2,4-dihydro-3H-1,2,4-triazole-3-thione, (1.058) 2-{{rel(2R,3S)-3-(2-chlorophenyl)-2-(2,4-difluorophenyl)oxiran-2-yl}methyl}-2,4-dihydro-3H-1,2,4-triazole-3-thione, (1.059) 5-(4-chlorobenzyl)-2-(chloromethyl)-2-methyl-1-(1H-1,2,4-triazol-1-yl)methyl)cyclopentanol, (1.060) 5-(allylsulfanyl)-1-{{3-(2-chlorophenyl)-2-(2,4-difluorophenyl)oxiran-2-yl}methyl}-1H-1,2,4-triazole, (1.061) 5-(allylsulfanyl)-1-{{rel(2R,3R)-3-(2-chlorophenyl)-2-(2,4-difluorophenyl)oxiran-2-yl}methyl}-1H-1,2,4-triazole, (1.062) 5-(allylsulfanyl)-1-{{rel(2R,3S)-3-(2-chlorophenyl)-2-(2,4-difluorophenyl)oxiran-2-yl}methyl}-1H-1,2,4-triazole, (1.063) N'-(2,5-dimethyl-4-{{3-(1,1,2,2-tetrafluoroethoxy)phenyl}sulfanyl}phenyl)-N-ethyl-N-methylimidoforamide, (1.064) N'-(2,5-dimethyl-4-{{3-(2,2,2-trifluoroethoxy)phenyl}sulfanyl}phenyl)-N-ethyl-N-methylimidoforamide, (1.065) N'-(2,5-dimethyl-4-{{3-(2,2,3,3-tetrafluoropropoxy)phenyl}sulfanyl}phenyl)-N-ethyl-N-methylimidoforamide, (1.066) N'-(2,5-dimethyl-4-{{3-(pentafluoroethoxy)phenyl}sulfanyl}phenyl)-N-ethyl-N-methylimidoforamide, (1.067) N'-(2,5-dimethyl-4-{{3-[(1,1,2,2-tetrafluoroethyl)sulfanyl]phenoxy}phenyl)-N-ethyl-N-methylimidoforamide, (1.068) N'-(2,5-dimethyl-4-{{3-[(2,2,2-trifluoroethyl)sulfanyl]phenoxy}phenyl)-N-ethyl-N-methylimidoforamide, (1.069) N'-(2,5-dimethyl-4-{{3-[(2,2,3,3-tetrafluoropropyl)sulfanyl]phenoxy}phenyl)-N-ethyl-N-methylimidoforamide, (1.070) N'-(2,5-dimethyl-4-{{3-[(pentafluoroethyl)sulfanyl]phenoxy}phenyl)-N-ethyl-N-methylimidoforamide, (1.071) N'-(2,5-

dimethyl-4-phenoxyphenyl)-N-ethyl-N-methylimidoforamide, (1.072) N'-(4-{3-(difluoromethoxy)phenyl}sulfanyl}-2,5-dimethylphenyl)-N-ethyl-N-methylimidoforamide, (1.073) N'-(4-{3-[(difluoromethyl)sulfanyl]phenoxy}-2,5-dimethylphenyl)-N-ethyl-N-methylimidoforamide, (1.074) N'-[5-bromo-6-(2,3-dihydro-1H-inden-2-yloxy)-2-methylpyridin-3-yl]-N-ethyl-N-methylimidoforamide, (1.075) N'-(4-[(4,5-dichloro-1,3-thiazol-2-yl)oxy]-2,5-dimethylphenyl)-N-ethyl-N-methylimidoforamide, (1.076) N'-(5-bromo-6-[(1R)-1-(3,5-difluorophenyl)ethoxy]-2-methylpyridin-3-yl)-N-ethyl-N-methylimidoforamide, (1.077) N'-(5-bromo-6-[(1S)-1-(3,5-difluorophenyl)ethoxy]-2-methylpyridin-3-yl)-N-ethyl-N-methylimidoforamide, (1.078) N'-(5-bromo-6-[(cis-4-isopropylcyclohexyl)oxy]-2-methylpyridin-3-yl)-N-ethyl-N-methylimidoforamide, (1.079) N'-(5-bromo-6-[(trans-4-isopropylcyclohexyl)oxy]-2-methylpyridin-3-yl)-N-ethyl-N-methylimidoforamide, (1.080) N'-(5-bromo-6-[1-(3,5-difluorophenyl)ethoxy]-2-methylpyridin-3-yl)-N-ethyl-N-methylimidoforamide, (1.081) Mefentrifluconazole, (1.082) Ipfentrifluconazole.

Preferably, a fungicide of this class is triadimenol which can be used both at the same time and sequentially with *P. lilacinum* 251.

2) Inhibitors of the respiratory chain at complex I or II, for example, (2.001) benzovindiflupyr, (2.002) bixafen, (2.003) boscalid, (2.004) carboxin, (2.005) fluopyram, (2.006) flutolanil, (2.007) fluxapyroxad, (2.008) furametpyr, (2.009) Isofetamid, (2.010) isopyrazam (anti-epimeric enantiomer 1R,4S,9S), (2.011) isopyrazam (anti-epimeric enantiomer 1S,4R,9R), (2.012) isopyrazam (anti-epimeric racemate 1RS,4SR,9SR), (2.013) isopyrazam (mixture of syn-epimeric racemate 1RS,4SR,9RS and anti-epimeric racemate 1RS,4SR,9SR), (2.014) isopyrazam (syn-epimeric enantiomer 1R,4S,9R), (2.015) isopyrazam (syn-epimeric enantiomer 1S,4R,9S), (2.016) isopyrazam (syn-epimeric racemate 1RS,4SR,9RS), (2.017) penflufen, (2.018) penthiopyrad, (2.019) pydiflumetofen, (2.020) Pyraziflumid, (2.021) sedaxane, (2.022) 1,3-dimethyl-N-(1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl)-1H-pyrazole-4-carboxamide, (2.023) 1,3-dimethyl-N-[(3R)-1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl]-1H-pyrazole-4-carboxamide, (2.024) 1,3-dimethyl-N-[(3S)-1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl]-1H-pyrazole-4-carboxamide, (2.025) 1-methyl-3-(trifluoromethyl)-N-[2'-(trifluoromethyl)biphenyl-2-yl]-1H-pyrazole-4-carboxamide, (2.026) 2-fluoro-6-(trifluoromethyl)-N-(1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl)benzamide, (2.027) 3-(difluoromethyl)-1-methyl-N-(1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl)-1H-pyrazole-4-carboxamide, (2.028) 3-(difluoromethyl)-1-methyl-N-[(3R)-1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl]-1H-pyrazole-4-carboxamide, (2.029) 3-(difluoromethyl)-1-methyl-N-[(3S)-1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl]-1H-pyrazole-4-carboxamide, (2.030) 3-(difluoromethyl)-N-(7-fluoro-1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl)-1-methyl-1H-pyrazole-4-carboxamide, (2.031) 3-(difluoromethyl)-N-[(3R)-7-fluoro-1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl]-1-methyl-1H-pyrazole-4-carboxamide, (2.032) 3-(difluoromethyl)-N-[(3S)-7-

fluoro-1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl]-1-methyl-1H-pyrazole-4-carboxamide, (2.033) 5,8-difluoro-N-[2-(2-fluoro-4-{[4-(trifluoromethyl)pyridin-2-yl]oxy}phenyl)ethyl]quinazolin-4-amine, (2.034) N-(2-cyclopentyl-5-fluorobenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide, (2.035) N-(2-tert-butyl-5-methylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide, (2.036) N-(2-tert-butylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide, (2.037) N-(5-chloro-2-ethylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide, (2.038) N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide, (2.039) N-[(1R,4S)-9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide, (2.040) N-[(1S,4R)-9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide, (2.041) N-[1-(2,4-dichlorophenyl)-1-methoxypropan-2-yl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide, (2.042) N-[2-chloro-6-(trifluoromethyl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide, (2.043) N-[3-chloro-2-fluoro-6-(trifluoromethyl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide, (2.044) N-[5-chloro-2-(trifluoromethyl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide, (2.045) N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-N-[5-methyl-2-(trifluoromethyl)benzyl]-1H-pyrazole-4-carboxamide, (2.046) N-cyclopropyl-3-(difluoromethyl)-5-fluoro-N-(2-fluoro-6-isopropylbenzyl)-1-methyl-1H-pyrazole-4-carboxamide, (2.047) N-cyclopropyl-3-(difluoromethyl)-5-fluoro-N-(2-isopropyl-5-methylbenzyl)-1-methyl-1H-pyrazole-4-carboxamide, (2.048) N-cyclopropyl-3-(difluoromethyl)-5-fluoro-N-(2-isopropylbenzyl)-1-methyl-1H-pyrazole-4-carbothioamide, (2.049) N-cyclopropyl-3-(difluoromethyl)-5-fluoro-N-(2-isopropylbenzyl)-1-methyl-1H-pyrazole-4-carboxamide, (2.050) N-cyclopropyl-3-(difluoromethyl)-5-fluoro-N-(5-fluoro-2-isopropylbenzyl)-1-methyl-1H-pyrazole-4-carboxamide, (2.051) N-cyclopropyl-3-(difluoromethyl)-N-(2-ethyl-4,5-dimethylbenzyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide, (2.052) N-cyclopropyl-3-(difluoromethyl)-N-(2-ethyl-5-fluorobenzyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide, (2.053) N-cyclopropyl-3-(difluoromethyl)-N-(2-ethyl-5-methylbenzyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide, (2.054) N-cyclopropyl-N-(2-cyclopropyl-5-fluorobenzyl)-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide, (2.055) N-cyclopropyl-N-(2-cyclopropyl-5-methylbenzyl)-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide, (2.056) N-cyclopropyl-N-(2-cyclopropylbenzyl)-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide.

Preferably, a fungicide of this class is selected from fluopyram and fluxapyroxad which can be used both at the same time and sequentially with *P. lilacinum* 251.

3) Inhibitors of the respiratory chain at complex III, for example, (3.001) ametoctradin, (3.002) amisulbrom, (3.004) coumethoxystrobin, (3.005) coumoxystrobin, (3.006) cyazofamid, (3.007) dimoxystrobin, (3.008) enoxastrobin, (3.009) famoxadone, (3.010) fenamidone, (3.011) flufenoxystrobin, (3.012) fluoxastrobin, (3.013) kresoxim-methyl, (3.014) metominostrobin, (3.015) orysastrobin, (3.016) picoxystrobin, (3.017) 5 pyraclostrobin, (3.018) pyrametostrobin, (3.019) pyraoxystrobin, (3.020) trifloxystrobin, (3.021) (2E)-2-{2-[[{[(1E)-1-(3-{[(E)-1-fluoro-2-phenylvinyl]oxy}phenyl)ethylidene]amino}oxy)methyl]phenyl}-2-(methoxyimino)-N-methylacetamide, (3.022) (2E,3Z)-5-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy}-2-(methoxyimino)-N,3-dimethylpent-3-enamide, (3.023) (2R)-2-{2-[(2,5-dimethylphenoxy)methyl]phenyl}-2-methoxy-N-methylacetamide, (3.024) (2S)-2-{2-[(2,5-dimethylphenoxy)methyl]phenyl}-2-methoxy-N-methylacetamide, (3.025) (3S,6S,7R,8R)-8-benzyl-3-[[3-[(isobutyryloxy)methoxy]-4-methoxypyridin-2-yl]carbonyl]amino]-6-methyl-4,9-dioxo-1,5-dioxonan-7-yl 2-methylpropanoate, (3.026) 2-{2-[(2,5-dimethylphenoxy)methyl]phenyl}-2-methoxy-N-methylacetamide, (3.027) N-(3-ethyl-3,5,5-trimethylcyclohexyl)-3-formamido-2-hydroxybenzamide, (3.028) (2E,3Z)-5-{[1-(4-chloro-2-fluorophenyl)-1H-pyrazol-3-yl]oxy}-2-(methoxyimino)-N,3-dimethylpent-3-enamide, (3.029) methyl {5-[3-(2,4-dimethylphenyl)-1H-pyrazol-1-yl]-2-methylbenzyl}carbamate.

Preferably, a fungicide of this class is trifloxystrobin which can be used both at the same time and sequentially with *P. lilacinum* 251.

4) Inhibitors of the mitosis and cell division, for example, (4.001) carbendazim, (4.002) diethofencarb, (4.003) ethaboxam, (4.004) fluopicolide, (4.005) pencycuron, (4.006) thiabendazole, (4.007) thiophanate-methyl, (4.008) zoxamide, (4.009) 3-chloro-4-(2,6-difluorophenyl)-6-methyl-5-phenylpyridazine, (4.010) 3-chloro-5-(4-chlorophenyl)-4-(2,6-difluorophenyl)-6-methylpyridazine, (4.011) 3-chloro-5-(6-chloropyridin-3-yl)-6-methyl-4-(2,4,6-trifluorophenyl)pyridazine, (4.012) 4-(2-bromo-4-fluorophenyl)-N-(2,6-difluorophenyl)-1,3-dimethyl-1H-pyrazol-5-amine, (4.013) 4-(2-bromo-4-fluorophenyl)-N-(2-bromo-6-fluorophenyl)-1,3-dimethyl-1H-pyrazol-5-amine, (4.014) 4-(2-bromo-4-fluorophenyl)-N-(2-bromophenyl)-1,3-dimethyl-1H-pyrazol-5-amine, (4.015) 4-(2-bromo-4-fluorophenyl)-N-(2-chloro-6-fluorophenyl)-1,3-dimethyl-1H-pyrazol-5-amine, (4.016) 4-(2-bromo-4-fluorophenyl)-N-(2-chlorophenyl)-1,3-dimethyl-1H-pyrazol-5-amine, (4.017) 4-(2-bromo-4-fluorophenyl)-N-(2-fluorophenyl)-1,3-dimethyl-1H-pyrazol-5-amine, (4.018) 4-(2-chloro-4-fluorophenyl)-N-(2,6-difluorophenyl)-1,3-dimethyl-1H-pyrazol-5-amine, (4.019) 4-(2-chloro-4-fluorophenyl)-N-(2-chloro-6-fluorophenyl)-1,3-dimethyl-1H-pyrazol-5-amine, (4.020) 4-(2-chloro-4-fluorophenyl)-N-(2-chlorophenyl)-1,3-dimethyl-1H-pyrazol-5-amine, (4.021) 4-(2-chloro-4-fluorophenyl)-N-(2-fluorophenyl)-1,3-dimethyl-1H-pyrazol-5-amine, (4.022) 4-(4-chlorophenyl)-5-(2,6-difluorophenyl)-3,6-dimethylpyridazine, (4.023) N-(2-bromo-6-fluorophenyl)-4-(2-chloro-4-fluorophenyl)-1,3-dimethyl-1H-pyrazol-5-amine, (4.024) N-(2-bromophenyl)-4-(2-chloro-4-fluorophenyl)-

1,3-dimethyl-1H-pyrazol-5-amine, (4.025) N-(4-chloro-2,6-difluorophenyl)-4-(2-chloro-4-fluorophenyl)-1,3-dimethyl-1H-pyrazol-5-amine.

5) Compounds capable to have a multisite action, for example, (5.001) bordeaux mixture, (5.002) captafol, (5.003) captan, (5.004) chlorothalonil, (5.005) copper hydroxide, (5.006) copper naphthenate, (5.007) copper oxide, (5.008) copper oxychloride, (5.009) copper(2+) sulfate, (5.010) dithianon, (5.011) dodine, (5.012) folpet, (5.014) maneb, (5.015) metiram, (5.016) metiram zinc, (5.017) oxine-copper, (5.018) propineb, (5.019) sulfur and sulfur preparations including calcium polysulfide, (5.020) thiram, (5.021) zineb, (5.022) ziram, (5.023) 6-ethyl-5,7-dioxo-6,7-dihydro-5H-pyrrolo[3',4':5,6][1,4]dithiino[2,3-c][1,2]thiazole-3-carbonitrile.

6) Compounds capable to induce a host defense, for example, (6.001) acibenzolar-S-methyl, (6.002) isotianil, (6.003) probenazole, (6.004) tiadinil.

7) Inhibitors of the amino acid and/or protein biosynthesis, for example, (7.001) cyprodinil, (7.002) kasugamycin, (7.003) kasugamycin hydrochloride hydrate, (7.004) oxytetracycline, (7.005) pyrimethanil, (7.006) 3-(5-fluoro-3,3,4,4-tetramethyl-3,4-dihydroisoquinolin-1-yl)quinoline.

8) Inhibitors of the ATP production, for example, (8.001) silthiofam.

9) Inhibitors of the cell wall synthesis, for example (9.001) benthiavalicarb, (9.002) dimethomorph, (9.003) flumorph, (9.004) iprovalicarb, (9.005) mandipropamid, (9.006) pyrimorph, (9.007) valifenalate, (9.008) (2E)-3-(4-tert-butylphenyl)-3-(2-chloropyridin-4-yl)-1-(morpholin-4-yl)prop-2-en-1-one, (9.009) (2Z)-3-(4-tert-butylphenyl)-3-(2-chloropyridin-4-yl)-1-(morpholin-4-yl)prop-2-en-1-one.

10) Inhibitors of the lipid and membrane synthesis, for example, (10.001) propamocarb, (10.002) propamocarb hydrochloride, (10.003) tolclofos-methyl.

Preferably, a fungicide of this class is selected from propamocarb and propamocarb hydrochloride which can be used both at the same time and sequentially with *P. lilacinum* 251.

11) Inhibitors of the melanin biosynthesis, for example (11.001) tricyclazole, (11.002) 2,2,2-trifluoroethyl {3-methyl-1-[(4-methylbenzoyl)amino]butan-2-yl} carbamate.

12) Inhibitors of the nucleic acid synthesis, for example, (12.001) benalaxyl, (12.002) benalaxyl-M (kiralaxyl), (12.003) metalaxyl, (12.004) metalaxyl-M (mefenoxam).

Preferably, a fungicide of this class is selected from metalaxyl and metalaxyl-M which can be used both at the same time and sequentially with *P. lilacinum* 251.

13) Inhibitors of the signal transduction, for example, (13.001) fludioxonil, (13.002) iprodione, (13.003) procymidone, (13.004) proquinazid, (13.005) quinoxifen, (13.006) vinclozolin.

14) Compounds capable to act as an uncoupler, for example (14.001) fluazinam, (14.002) meptyldinocap.

15) Further compounds, for example, (15.001) Absciscic acid, (15.002) benthiazole, (15.003) bethoxazin, (15.004) capsimycin, (15.005) carvone, (15.006) chinomethionat, (15.007) cufraneb, (15.008) cyflufenamid, (15.009) cymoxanil, (15.010) cyprosulfamide, (15.011) flutianil, (15.012) fosetyl-aluminium, (15.013) fosetyl-calcium, (15.014) fosetyl-sodium, (15.015) methyl isothiocyanate, (15.016) metrafenone, (15.017) mildiomycin, (15.018) natamycin, (15.019) nickel dimethyldithiocarbamate, (15.020) nitrothal-isopropyl, (15.021) oxamocarb, (15.022) Oxathiapiprolin, (15.023) oxyfenthiiin, (15.024) pentachlorophenol and salts, (15.025) phosphorous acid and its salts, (15.026) propamocarb-fosetylate, (15.027) pyriofenone (chlazafenone), (15.028) tebufloquin, (15.029) tecloftalam, (15.030) tolnifanide, (15.031) 1-(4-{4-[(5R)-5-(2,6-difluorophenyl)-4,5-dihydro-1,2-oxazol-3-yl]-1,3-thiazol-2-yl}piperidin-1-yl)-2-[5-methyl-3-(trifluoromethyl)-1H-pyrazol-1-yl]ethanone, (15.032) 1-(4-{4-[(5S)-5-(2,6-difluorophenyl)-4,5-dihydro-1,2-oxazol-3-yl]-1,3-thiazol-2-yl}piperidin-1-yl)-2-[5-methyl-3-(trifluoromethyl)-1H-pyrazol-1-yl]ethanone, (15.033) 2-(6-benzylpyridin-2-yl)quinazoline, (15.034) 2,6-dimethyl-1H,5H-[1,4]dithiino[2,3-c:5,6-c']dipyrrole-1,3,5,7(2H,6H)-tetrone, (15.035) 2-[3,5-bis(difluoromethyl)-1H-pyrazol-1-yl]-1-[4-(4-{5-[2-(prop-2-yn-1-yloxy)phenyl]-4,5-dihydro-1,2-oxazol-3-yl]-1,3-thiazol-2-yl}piperidin-1-yl)ethanone, (15.036) 2-[3,5-bis(difluoromethyl)-1H-pyrazol-1-yl]-1-[4-(4-{5-[2-chloro-6-(prop-2-yn-1-yloxy)phenyl]-4,5-dihydro-1,2-oxazol-3-yl]-1,3-thiazol-2-yl}piperidin-1-yl)ethanone, (15.037) 2-[3,5-bis(difluoromethyl)-1H-pyrazol-1-yl]-1-[4-(4-{5-[2-fluoro-6-(prop-2-yn-1-yloxy)phenyl]-4,5-dihydro-1,2-oxazol-3-yl]-1,3-thiazol-2-yl}piperidin-1-yl)ethanone, (15.038) 2-[6-(3-fluoro-4-methoxyphenyl)-5-methylpyridin-2-yl]quinazoline, (15.039) 2-[(5R)-3-[2-(1-{[3,5-bis(difluoromethyl)-1H-pyrazol-1-yl]acetyl}piperidin-4-yl)-1,3-thiazol-4-yl]-4,5-dihydro-1,2-oxazol-5-yl]-3-chlorophenyl methanesulfonate, (15.040) 2-[(5S)-3-[2-(1-{[3,5-bis(difluoromethyl)-1H-pyrazol-1-yl]acetyl}piperidin-4-yl)-1,3-thiazol-4-yl]-4,5-dihydro-1,2-oxazol-5-yl]-3-chlorophenyl methanesulfonate, (15.041) 2-{2-[(7,8-difluoro-2-methylquinolin-3-yl)oxy]-6-fluorophenyl}propan-2-ol, (15.042) 2-{2-fluoro-6-[(8-fluoro-2-methylquinolin-3-yl)oxy]phenyl}propan-2-ol, (15.043) 2-{3-[2-(1-{[3,5-bis(difluoromethyl)-1H-pyrazol-1-yl]acetyl}piperidin-4-yl)-1,3-thiazol-4-yl]-

4,5-dihydro-1,2-oxazol-5-yl}-3-chlorophenyl methanesulfonate, (15.044) 2-{3-[2-(1-[[3,5-bis(difluoromethyl)-1H-pyrazol-1-yl]acetyl]piperidin-4-yl)-1,3-thiazol-4-yl]-4,5-dihydro-1,2-oxazol-5-yl}phenyl methanesulfonate, (15.045) 2-phenylphenol and salts, (15.046) 3-(4,4,5-trifluoro-3,3-dimethyl-3,4-dihydroisoquinolin-1-yl)quinoline, (15.047) 3-(4,4-difluoro-3,3-dimethyl-3,4-dihydroisoquinolin-1-yl)quinoline, (15.048) 4-amino-5-fluoropyrimidin-2-ol (tautomeric form: 4-amino-5-fluoropyrimidin-2(1H)-one), (15.049) 4-oxo-4-[(2-phenylethyl)amino]butanoic acid, (15.050) 5-amino-1,3,4-thiadiazole-2-thiol, (15.051) 5-chloro-N'-phenyl-N'-(prop-2-yn-1-yl)thiophene-2-sulfonohydrazide, (15.052) 5-fluoro-2-[(4-fluorobenzyl)oxy]pyrimidin-4-amine, (15.053) 5-fluoro-2-[(4-methylbenzyl)oxy]pyrimidin-4-amine, (15.054) 9-fluoro-2,2-dimethyl-5-(quinolin-3-yl)-2,3-dihydro-1,4-benzoxazepine, (15.055) but-3-yn-1-yl {6-[[[(Z)-(1-methyl-1H-tetrazol-5-yl)(phenyl)methylene]amino]oxy)methyl]pyridin-2-yl}carbamate, (15.056) ethyl (2Z)-3-amino-2-cyano-3-phenylacrylate, (15.057) phenazine-1-carboxylic acid, (15.058) propyl 3,4,5-trihydroxybenzoate, (15.059) quinolin-8-ol, (15.060) quinolin-8-ol sulfate (2:1), (15.061) tert-butyl {6-[[[(1-methyl-1H-tetrazol-5-yl)(phenyl)methylene]amino]oxy)methyl]pyridin-2-yl}carbamate, (15.062) 5-fluoro-4-imino-3-methyl-1-[(4-methylphenyl)sulfonyl]-3,4-dihydropyrimidin-2(1H)-one.

15 Preferably, a fungicide of this class is fosetyl-aluminum which can be used both at the same time and sequentially with *P. lilacinum* 251.

Insecticides may be of the following classes:

20 (1) Acetylcholinesterase (AChE) inhibitors, for example, carbamates, e.g., Alanycarb, Aldicarb, Bendiocarb, Benfuracarb, Butocarboxim, Butoxycarboxim, Carbaryl, Carbofuran, Carbosulfan, Ethiofencarb, Fenobucarb, Formetanate, Furathiocarb, Isoprocarb, Methiocarb, Methomyl, Metolcarb, Oxamyl, Pirimicarb, Propoxur, Thiodicarb, Thiofanox, Triazamate, Trimethacarb, XMC and Xylylcarb or  
 25 organophosphates, e.g., Acephate, Azamethiphos, Azinphos-ethyl, Azinphos-methyl, Cadusafos, Chlorethoxyfos, Chlorfenvinphos, Chlormephos, Chlorpyrifos, Chlorpyrifos-methyl, Coumaphos, Cyanophos, Demeton-S-methyl, Diazinon, Dichlorvos/DDVP, Dicrotophos, Dimethoate, Dimethylvinphos, Disulfoton, EPN, Ethion, Ethoprophos, Famphur, Fenamiphos, Fenitrothion, Fenthion, Fosthiazate, Heptenophos, Imicyafos, Isofenphos, Isopropyl O-(methoxyaminothio-phosphoryl)salicylate, Isoxathion,  
 30 Malathion, Mecarbam, Methamidophos, Methidathion, Mevinphos, Monocrotophos, Naled, Omethoate, Oxydemeton-methyl, Parathion, Parathion-methyl, Phenthoate, Phorate, Phosalone, Phosmet, Phosphamidon, Phoxim, Pirimiphos-methyl, Profenofos, Propetamphos, Prothiofos, Pyraclofos, Pyridaphenthion, Quinalphos, Sulfotep, Tebupirimfos, Temephos, Terbufos, Tetrachlorvinphos, Thiometon, Triazophos, Trichlorfon and Vamidotion.

35

- (2) GABA-gated chloride channel antagonists, for example cyclodiene organochlorines, e.g., Chlordane and Endosulfan, or phenylpyrazoles (fiproles), e.g., Ethiprole and Fipronil.
- (3) Sodium channel modulators / voltage-dependent sodium channel blockers, for example, pyrethroids, e.g., Acrinathrin, Allethrin, d-cis-trans Allethrin, d-trans Allethrin, Bifenthrin, Bioallethrin, Bioallethrin S-cyclopentenyl isomer, Bioresmethrin, Cycloprothrin, Cyfluthrin, beta-Cyfluthrin, Cyhalothrin, lambda-Cyhalothrin, gamma-Cyhalothrin, Cypermethrin, alpha-Cypermethrin, beta-Cypermethrin, theta-Cypermethrin, zeta-Cypermethrin, Cyphenothrin [(1R)-trans isomers], Deltamethrin, Empenthrin [(EZ)-(1R) isomers), Esfenvalerate, Etofenprox, Fenpropathrin, Fenvalerate, Flucythrinate, Flumethrin, tau-Fluvalinate, Halfenprox, Imiprothrin, Kadethrin, Momfluorothrin, Permethrin, Phenothrin [(1R)-trans isomer), Prallethrin, Pyrethrine (pyrethrum), Resmethrin, Silafluofen, Tefluthrin, Tetramethrin, Tetramethrin [(1R) isomers)], Tralomethrin and Transfluthrin or DDT or Methoxychlor.
- (4) Nicotinic acetylcholine receptor (nAChR) agonists, for example, neonicotinoids, e.g., Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Nitenpyram, Thiacloprid and Thiamethoxam or Nicotine or Sulfoxaflor or Flupyrifadafurone.
- (5) Nicotinic acetylcholine receptor (nAChR) allosteric activators, for example, spinosyns, e.g., Spinetoram and Spinosad.
- (6) Chloride channel activators, for example, avermectins/milbemycins, e.g., Abamectin, Emamectin benzoate, Lepimectin and Milbemectin.
- (7) Juvenile hormone mimics, for example, juvenile hormone analogues, e.g., Hydroprene, Kinoprene and Methoprene or Fenoxycarb or Pyriproxyfen.
- (8) Miscellaneous non-specific (multi-site) inhibitors, for example, alkyl halides, e.g., Methyl bromide and other alkyl halides; or Chloropicrin or Sulfuryl fluoride or Borax or Tartar emetic.
- (9) Selective homopteran feeding blockers, e.g., Pymetrozine or Flonicamid.
- (10) Mite growth inhibitors, e.g., Clofentezine, Hexythiazox and Diflovidazin or Etoxazole.
- (11) Microbial disruptors of insect midgut membranes, e.g., *Bacillus thuringiensis* subspecies *israelensis*, *Bacillus sphaericus*, *Bacillus thuringiensis* subspecies *aizawai*, *Bacillus thuringiensis* subspecies *kurstaki*,

*Bacillus thuringiensis* subspecies *tenebrionis* and BT crop proteins: Cry1Ab, Cry1Ac, Cry1Fa, Cry2Ab, mCry3A, Cry3Ab, Cry3Bb, Cry34/35Ab1.

5 (12) Inhibitors of mitochondrial ATP synthase, for example, Diafenthiuron or organotin miticides, e.g., Azocyclotin, Cyhexatin and Fenbutatin oxide or Propargite or Tetradifon.

(13) Uncouplers of oxidative phosphorylation via disruption of the proton gradient, for example, Chlorfenapyr, DNOC and Sulfluramid.

10 (14) Nicotinic acetylcholine receptor (nAChR) channel blockers, for example, Bensultap, Cartap hydrochloride, Thiocyclam and Thiosultap-sodium.

(15) Inhibitors of chitin biosynthesis, type 0, for example, Bistrifluron, Chlorfluazuron, Diflubenzuron, Flucycloxuron, Flufenoxuron, Hexaflumuron, Lufenuron, Novaluron, Noviflumuron, Teflubenzuron and  
15 Triflumuron.

(16) Inhibitors of chitin biosynthesis, type 1, for example, Buprofezin.

(17) Moulting disruptors, for example, Cyromazine.  
20

(18) Ecdysone receptor agonists, for example, Chromafenozide, Halofenozide, Methoxyfenozide and Tebufenozide.

(19) Octopamine receptor agonists, for example, Amitraz.  
25

(20) Mitochondrial complex III electron transport inhibitors, for example, Hydramethylnon or Acequinocyl or Fluacrypyrim.

(21) Mitochondrial complex I electron transport inhibitors, for example, METI acaricides, e.g., Fenazaquin,  
30 Fenpyroximate, Pyrimidifen, Pyridaben, Tebufenpyrad and Tolfenpyrad or Rotenone (Derris).

(22) Voltage-dependent sodium channel blockers, e.g., Indoxacarb or Metaflumizone.

(23) Inhibitors of acetyl CoA carboxylase, for example, tetrionic and tetramic acid derivatives, e.g.,  
35 Spirobudiclofen, Spirodiclofen, Spiromesifen and Spirotetramat.

- (24) Mitochondrial complex IV electron transport inhibitors, for example, phosphines, e.g., Aluminium phosphide, Calcium phosphide, Phosphine and Zinc phosphide or Cyanide.
- 5 (25) Mitochondrial complex II electron transport inhibitors, for example, Cyenopyrafen and Cyflumetofen.
- (26) Ryanodine receptor modulators, for example diamides, e.g., Chlorantraniliprole, Cyantraniliprole, Flubendiamide and Tetrachloroantraniliprole.
- 10 Further active ingredients with unknown or uncertain mode of action, for example, Afidopyropen, Afoxolaner, Azadirachtin, Benclouthiaz, Benzoximate, Bifenazate, Broflanilide, Bromopropylate, Chinomethionat, Cryolite, Cyclaniliprole, Cycloxaprid, Cyhalodiamide Dicloromezotiaz, Dicofol, Diflovidazin, Flometoquin, Fluazaindolizine, Fluensulfone, Flufenerim, Flufenoxystrobin, Flufiprole, Fluhexafon, Fluopyram, Fluralaner, Fluxametamide, Fufenozide, Guadipyr, Heptafluthrin, Imidaclathiz,
- 15 Iprodione, Lotilaner, Meperfluthrin, Paichongding, Pyflubumide, Pyridalyl, Pyrifluquinazon, Pymimostrobin, Sarolaner, Tetramethylfluthrin, Tetrilaniliprole, Tetrachlorantraniliprole, Tioxazafen, Thiofluoximate, Triflumezopyrim and Iodomethane; furthermore products based on *Bacillus firmus* (including but not limited to strain CNCM I-1582, such as, for example, VOTIVO<sup>®</sup>, BIONEM<sup>®</sup>) or one of the following known active compounds: 1-{2-fluoro-4-methyl-5-[(2,2,2-trifluorethyl)sulfinyl]phenyl}-3-
- 20 (trifluoromethyl)-1H-1,2,4-triazol-5-amine (known from WO2006/043635), {1'-[(2E)-3-(4-chlorophenyl)prop-2-en-1-yl]-5-fluorospiro[indole-3,4'-piperidin]-1(2H)-yl}(2-chloropyridin-4-yl)methanone (known from WO 2003/106457), 2-chloro-N-[2-{1-[(2E)-3-(4-chlorophenyl)prop-2-en-1-yl]piperidin-4-yl}-4-(trifluoromethyl)phenyl]isonicotinamide (known from WO2006/003494), 3-(2,5-dimethylphenyl)-4-hydroxy-8-methoxy-1,8-diazaspiro[4.5]dec-3-en-2-one (known from WO 2009/049851),
- 25 3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1,8-diazaspiro[4.5]dec-3-en-4-yl ethyl carbonate (known from WO 2009/049851), 4-(but-2-yn-1-yloxy)-6-(3,5-dimethylpiperidin-1-yl)-5-fluoropyrimidine (known from WO2004/099160), 4-(but-2-yn-1-yloxy)-6-(3-chlorophenyl)pyrimidine (known from WO 2003/076415), PF1364 (CAS-Reg. No. 1204776-60-2), methyl 2-[2-({[3-bromo-1-(3-chloropyridin-2-yl)-1H-pyrazol-5-yl]carbonyl}amino)-5-chloro-3-methylbenzoyl]-2-methylhydrazinecarboxylate (known from WO
- 30 2005/085216), methyl 2-[2-({[3-bromo-1-(3-chloropyridin-2-yl)-1H-pyrazol-5-yl]carbonyl}amino)-5-cyano-3-methylbenzoyl]-2-ethylhydrazinecarboxylate (known from WO 2005/085216), methyl 2-[2-({[3-bromo-1-(3-chloropyridin-2-yl)-1H-pyrazol-5-yl]carbonyl}amino)-5-cyano-3-methylbenzoyl]-2-
- methylhydrazinecarboxylate (known from WO 2005/085216), methyl 2-[3,5-dibromo-2-({[3-bromo-1-(3-chloropyridin-2-yl)-1H-pyrazol-5-yl]carbonyl}amino)benzoyl]-2-ethylhydrazinecarboxylate (known from
- 35 WO 2005/085216), , N-[2-(5-amino-1,3,4-thiadiazol-2-yl)-4-chloro-6-methylphenyl]-3-bromo-1-(3-

chloropyridin-2-yl)-1H-pyrazole-5-carboxamide (known from CN 102057925), 8-chloro-N-[(2-chloro-5-methoxyphenyl)sulfonyl]-6-(trifluoromethyl)imidazo[1,2-a]pyridine-2-carboxamide (known from WO 2009/080250), N-[(2E)-1-[(6-chloropyridin-3-yl)methyl]pyridin-2(1H)-ylidene]-2,2,2-trifluoroacetamide (known from WO 2012/029672 ), 1-[(2-chloro-1,3-thiazol-5-yl)methyl]-4-oxo-3-phenyl-4H-pyrido[1,2-a]pyrimidin-1-ium-2-olate (known from WO 2009/099929 ), 1-[(6-chloropyridin-3-yl)methyl]-4-oxo-3-phenyl-4H-pyrido[1,2-a]pyrimidin-1-ium-2-olate (known from WO 2009/099929 ), 4-(3-{2,6-dichloro-4-[(3,3-dichloroprop-2-en-1-yl)oxy]phenoxy}propoxy)-2-methoxy-6-(trifluoromethyl)pyrimidine (known from CN 101337940), N-[2-(tert-butylcarbonyl)-4-chloro-6-methylphenyl]-1-(3-chloropyridin-2-yl)-3-(fluoromethoxy)-1H-pyrazole-5-carboxamide (known from WO 2008/134969), butyl [2-(2,4-dichlorophenyl)-3-oxo-4-oxaspiro[4.5]dec-1-en-1-yl] carbonate (known from CN 102060818), 3E)-3-[1-[(6-chloro-3-pyridyl)methyl]-2-pyridylidene]-1,1,1-trifluoro-propan-2-one (known from WO 2013/144213), N-(methylsulfonyl)-6-[2-(pyridin-3-yl)-1,3-thiazol-5-yl]pyridine-2-carboxamide (known from WO 2012/000896), N-[3-(benzylcarbonyl)-4-chlorophenyl]-1-methyl-3-(pentafluoroethyl)-4-(trifluoromethyl)-1H-pyrazole-5-carboxamide (known from WO 2010/051926), 5-bromo-4-chloro-N-[4-chloro-2-methyl-6-(methylcarbonyl)phenyl]-2-(3-chloro-2-pyridyl)pyrazole-3-carboxamido (known from CN 103232431), , Tioxazafen, 4-[5-(3,5-dichlorophenyl)-4,5-dihydro-5-(trifluoromethyl)-3-isoxazolyl]-2-methyl-N-(*cis*-1-oxido-3-thietanyl)-benzamide, 4-[5-(3,5-dichlorophenyl)-4,5-dihydro-5-(trifluoromethyl)-3-isoxazolyl]-2-methyl-N-(*trans*-1-oxido-3-thietanyl)-benzamide and 4-[(5S)-5-(3,5-dichlorophenyl)-4,5-dihydro-5-(trifluoromethyl)-3-isoxazolyl]-2-methyl-N-(*cis*-1-oxido-3-thietanyl)benzamide (known from WO 2013/050317 A1), N-[3-chloro-1-(3-pyridinyl)-1H-pyrazol-4-yl]-N-ethyl-3-[(3,3,3-trifluoropropyl)sulfinyl]-propanamide, (+)-N-[3-chloro-1-(3-pyridinyl)-1H-pyrazol-4-yl]-N-ethyl-3-[(3,3,3-trifluoropropyl)sulfinyl]-propanamide and (-)-N-[3-chloro-1-(3-pyridinyl)-1H-pyrazol-4-yl]-N-ethyl-3-[(3,3,3-trifluoropropyl)sulfinyl]-propanamide (known from WO 2013/162715A2, WO 2013/162716 A2, US 2014/0213448 A1), 5-[[2E)-3-chloro-2-propen-1-yl]amino]-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile (known from CN 101337937 A), 3-bromo-N-[4-chloro-2-methyl-6-[(methylamino)thioxomethyl]phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide, (Liudaibenjiaxuanan, known from CN 103109816 A); N-[4-chloro-2-[(1,1-dimethylethyl)amino]carbonyl]-6-methylphenyl]-1-(3-chloro-2-pyridinyl)-3-(fluoromethoxy)-1H-Pyrazole-5-carboxamide (known from WO 2012034403 A1), N-[2-(5-amino-1,3,4-thiadiazol-2-yl)-4-chloro-6-methylphenyl]-3-bromo-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide (known from WO 2011/085575 A1), 4-[3-[2,6-dichloro-4-[(3,3-dichloro-2-propen-1-yl)oxy]phenoxy]propoxy]-2-methoxy-6-(trifluoromethyl)-pyrimidine (known from CN 101337940 A); (2E)- and 2(Z)-2-[2-(4-cyanophenyl)-1-[3-(trifluoromethyl)phenyl]ethylidene]-N-[4-(difluoromethoxy)phenyl]-hydrazinecarboxamide (known from CN 101715774 A); 3-(2,2-dichloroethenyl)-2,2-dimethyl-4-(1H-benzimidazol-2-yl)phenyl-cyclopropanecarboxylic acid ester (known from CN 103524422 A); (4aS)-7-chloro-2,5-dihydro-2-[[[(methoxycarbonyl)4-[(trifluoromethyl)thio]

phenyl]amino]carbonyl]-indeno[1,2-*e*][1,3,4]oxadiazine-4a(3*H*)-carboxylic acid methyl ester (known from CN 102391261 A).

5 Nematicides comprise dichlorpropene, metam sodium, metam potassium, chloropicrin, oxamyl, carbofuran, cleothocarb, fosthiazate, aldicarb, aldoxycarb, fenamiphos, cadusaphos, abamectin, cyanamide, dazomet, methyl-bromide, terbufos, ethoprophos, ethylene-dibromide, phorate, methyl isothiocyanate, thiodicarb, sodium tetrathiocarbonate, tioxazafen, iprodione, fluensulfone, imicyafos, mimethyl-disulfide, spirotetramate, fluazaindolizine, ebufos, isazafos, fensulfothion, thionazin and fluopyram. Fumigants are generally only suitable for sequential application in connection with *P. lilacinum*.

10 Plant protection agents may also comprise biological control agents. Preferably, the biological control agent has nematicidal, fungicidal or insecticidal properties or a beneficial effect on plant health.

Also fungicidally, insecticidally, nematicidally active biological control agents as well as those having an effect on plant health promotion may be used.

The term “at least one” indicates that in any case one further plant protection agent is applied in addition to *Purpureocillium lilacinum* or its spores. However, more than one such as (at least) two, (at least) three, (at least) four, (at least) 5 or even more further plant protection agents may be applied according to the present embodiment.

In a more preferred embodiment, said at least one further plant protection agent is selected from the group consisting of fluopyram, *B. firmus* strain CNCM I-1582 (also known as VOTIVO®), *B. subtilis*, in particular strain QST713 (disclosed in e.g., U.S. Patent Nos. 6,060,051; 6,103,228; 6,291,426; 6,417,163; and 25 6,638,910), abamectin, aldicarb, aldoxycarb, carbofuran, cleothocarb, fenamiphos, fluensulfone, fluazaindolizine, oxamyl, fosthiazate, tioxazafen, iprodione, ethoprophos, flupyradifurone, tetraniliprole, rynaxypyr, cyazypyr, thiomethoxam, clothianidin, imidacloprid, thiacloprid, acetamiprid, sulfoxaflor and thiodicarb. Plant protection agents to be combined with *P. lilacinum* which are biological control agents comprise microorganisms or spores thereof of e.g., *Trichoderma harzianum* strain T-22, but also beneficial 30 nematodes such as *Steinernema feltiae*, *Heterorhabditis bacteriophora*, and *Steinernema carpocapsae*. In one embodiment, *P. lilacinum* or spores thereof are applied simultaneously or sequentially with *B. firmus* strain CNCM I-1582 and clothianidin (available as Poncho/VoTiVO from Bayer CropScience).

Accordingly, in order to achieve a better PGP or plant health effect, it is particularly advantageous to 35 sequentially apply *P. lilacinum* and fluopyram. Here, it is most useful to have *P. lilacinum* applied as last

PGP or plant health promoting agent, also in order to reduce residues in the harvested crop. This does, however, not exclude that *P. lilacinum* may also and in addition be applied prior to application of fluopyram.

- 5 If needed, *P. lilacinus* may also be applied together with certain other fungicides where compatibility has been shown, such as fosetyl-Al, trifloxystrobin, metalaxyl, pentachloronitrobenzene, fluxapyroxad, propamocarb and triadimenol. Compatible insecticides and/or nematicides comprise carbofuran, cadusafos, fenamiphos, furfural, terbufos, tioxazafen, fluazaindolizine, fosthiazate, flupyradifurone, imidacloprid, bifenthrin.

- 10 Besides biological and chemical plant protection agents, technical methods are commonly applied by farmers to control effectively soilborne pathogens as well as nematodes. Solarization by using radiant heat from the sun trapped by clear polyethylene mulch or tarp throughout several weeks increases soil temperature thus act as the lethal agent, respectively. All such methods may also be applied in conjunction  
15 with the present invention.

- The present invention may be applied to any agricultural plant. Plants are understood here to mean all plants and plant populations such as desired wild plants or crop plants (including naturally occurring crop plants). Crop plants may be plants which can be obtained by conventional breeding and optimization  
20 methods which can be assisted or supplemented by one or more biotechnological methods such as by use of double haploids, protoplast fusion, random and directed mutagenesis, molecular or genetic markers or by bioengineering and genetic engineering methods or combinations of these methods, including transgenic plants, plants modified by directed genome engineering such as by the use of zinc finger nucleases, meganucleases, TALE nucleases or CRISPR/Cas9 and including the plant cultivars which can or cannot be  
25 protected by plant breeders' certificates.

- The crops to be treated, which have only been described in a general manner, are differentiated and specified below and include vegetables, perennial crops, ornamentals, spices and cereals. Thus, with regard to use, vegetables are understood to mean, for example, fruit vegetables and flower-heads as vegetables, for  
30 example carrots, bell peppers, chili peppers, tomatoes, aubergines, cucumbers, cucurbits, courgettes, broad beans, runner beans, bush beans, peas, artichokes, maize; but also leafy vegetables, for example lettuce, chicory, endives, cress, rocket salad, field salad, iceberg lettuce, leek, spinach, swiss chard; additionally tuber vegetables, root vegetables and stem vegetables, for example celeriac, beetroot, carrots, garden radish, horseradish, salsify, asparagus, table beet, palm shoots, bamboo shoots, and also bulb vegetables, for

example onions, leek, fennel, garlic; additionally brassica vegetables, such as cauliflower, broccoli, kohlrabi, red cabbage, white cabbage, green cabbage, savoy cabbage, brussels sprouts, chinese cabbage.

In this regard, perennial crops are understood to mean citrus fruit, for example oranges, grapefruit, 5 mandarins, lemons, limes, bitter oranges, kumquats, satsumas; but also pome fruit, for example apples, pears and quince, and stone fruit, for example peaches, nectarines, cherries, plums, common plums, apricots; additionally grapevine, hops, olives, tea, soya, oilseed rape, cotton, sugar cane, beet, potatoes, tobacco and tropical crops, for example mangoes, papayas, figs, pineapples, dates, bananas, durians, kakis, coconuts, cacao, coffee, avocados, lychees, maracujas, guavas, and also almonds and nuts, for example hazelnuts, 10 walnuts, pistachios, cashew nuts, brazil nuts, pecan nuts, butter nuts, chestnuts, hickory nuts, macadamia nuts, peanuts, and additionally also soft fruit, for example blackcurrants, gooseberries, raspberries, blackberries, blueberries, strawberries, red bilberries, kiwis and cranberries.

Ornamental plants are understood to mean annual and perennial plants, for example, flowers to be cut from 15 plants, for example, roses, carnations, gerbera, lilies, marguerites, chrysanthemums, tulips, daffodils, anemones, poppies, amaryllis, dahlias, azaleas, malves, but also, for example, bedding plants, potted plants and shrubs, for example roses, tagetes, pansies, geraniums, fuchsias, hibiscus, chrysanthemums, busy lizzies, cyclamen, african violets, sunflowers, begonias, in ornamental lawns, in golf lawns, but also in cereals such as barley, wheat, rye, triticale, oats, in rice, in millet, in maize, additionally, for example, 20 bushes and conifers, for example, fig trees, rhododendron, spruce trees, fir trees, pine trees, yew trees, juniper trees, stone pines, rose bays.

Spices are understood to mean annual and perennial plants, for example, aniseed, chili pepper, bell pepper, 25 pepper, vanilla, marjoram, thyme, cloves, juniper berries, cinnamon, tarragon, coriander, saffron, ginger.

Preferred plants are selected from the group consisting of soybean, corn, wheat, triticale, barley, oat, rye, rape, millet, rice, sunflower, cotton, sugar beet, pome fruit, stone fruit, citrus, banana, strawberry, blueberry, almond, grape, mango, papaya, peanut, potato, tomato, pepper, cucurbit, cucumber, melon, watermelon, garlic, onion, broccoli, carrot, cabbage, bean, dry bean, canola, pea, lentil, alfalfa, trefoil, clover, flax, 30 elephant grass, grass, lettuce, sugarcane, tea, tobacco and coffee, nuts; each in its natural or genetically modified form.

In a further preferred embodiment, transgenic plants, and plant cultivars which have been obtained by genetic engineering methods, if appropriate in combination with conventional methods (Genetically 35 Modified Organisms), and parts thereof are treated.

The method according to the invention can be used in the treatment of genetically modified organisms (GMOs), e.g., plants or seeds. Genetically modified plants (or transgenic plants) are plants of which a heterologous gene has been stably integrated into the genome. The expression "heterologous gene" essentially means a gene which is provided or assembled outside the plant and when introduced in the nuclear, chloroplastic or mitochondrial genome gives the transformed plant new or improved agronomic or other properties by expressing a protein or polypeptide of interest or by downregulating or silencing other gene(s) which are present in the plant (using, for example, antisense technology, cosuppression technology or RNA interference-RNAi-technology). A heterologous gene that is located in the genome is also called a transgene. A transgene that is defined by its particular location in the plant genome is called a transformation event or transgenic event. Exemplary genetically modified plants are disclosed e.g., in US 2014/005047 in a non-limiting fashion.

It is more preferred that the agricultural plant is tomato, cucumber, corn, soy, ornamentals, coffee, carrots, potato or grapevine. It is even more preferred that the agricultural plant is selected from tomato, cucumber and corn.

Examples of typical formulations include water-soluble liquids (SL), emulsifiable concentrates (EC), emulsions in water (EW), suspension concentrates (SC, SE, FS, OD), water-dispersible granules (WG), granules (GR) and capsule concentrates (CS); these and other possible types of formulation are described, for example, by Crop Life International and in Pesticide Specifications, Manual on Development and Use of FAO and WHO Specifications for Pesticides, FAO Plant Production and Protection Papers – 173, prepared by the FAO/WHO Joint Meeting on Pesticide Specifications, 2004, ISBN: 9251048576. The formulations may comprise active agrochemical compounds other than one or more active compounds of the invention.

The formulations or application forms in question preferably comprise auxiliaries, such as extenders, solvents, spontaneity promoters, carriers, emulsifiers, dispersants, frost protectants, biocides, thickeners and/or other auxiliaries, such as adjuvants, for example. An adjuvant in this context is a component which enhances the biological effect of the formulation, without the component itself having a biological effect. Examples of adjuvants are agents which promote the retention, spreading, attachment to the leaf surface, or penetration.

These formulations are produced in a known manner, for example by mixing the active compounds with auxiliaries such as, for example, extenders, solvents and/or solid carriers and/or further auxiliaries, such as, for example, surfactants. The formulations are prepared either in suitable plants or else before or during the application.

Suitable for use as auxiliaries are substances which are suitable for imparting to the formulation of the active compound or the application forms prepared from these formulations (such as, e.g., usable crop protection agents, such as spray liquors or seed dressings) particular properties such as certain physical, technical and/or biological properties.

Suitable extenders are, for example, water, polar and nonpolar organic chemical liquids, for example from the classes of the aromatic and non-aromatic hydrocarbons (such as paraffins, alkylbenzenes, alkylnaphthalenes, chlorobenzenes), the alcohols and polyols (which, if appropriate, may also be substituted, etherified and/or esterified), the ketones (such as acetone, cyclohexanone), esters (including fats and oils) and (poly)ethers, the unsubstituted and substituted amines, amides, lactams (such as N-alkylpyrrolidones) and lactones, the sulphones and sulphoxides (such as dimethyl sulphoxide).

If the extender used is water, it is also possible to employ, for example, organic solvents as auxiliary solvents. Essentially, suitable liquid solvents are: aromatics such as xylene, toluene or alkylnaphthalenes, chlorinated aromatics and chlorinated aliphatic hydrocarbons such as chlorobenzenes, chloroethylenes or methylene chloride, aliphatic hydrocarbons such as cyclohexane or paraffins, for example petroleum fractions, mineral and vegetable oils, alcohols such as butanol or glycol and also their ethers and esters, ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone or cyclohexanone, strongly polar solvents such as dimethylformamide, dimethylacetamide and dimethyl sulphoxide, and also water. Preferred auxiliary solvents are selected from the group consisting of acetone and N,N'-dimethylacetamide.

In principle it is possible to use all suitable and compatible solvents. Suitable solvents are, for example, aromatic hydrocarbons, such as xylene, toluene or alkylnaphthalenes, for example, chlorinated aromatic or aliphatic hydrocarbons, such as chlorobenzene, chloroethylene or methylene chloride, for example, aliphatic hydrocarbons, such as cyclohexane, for example, paraffins, petroleum fractions, mineral and vegetable oils, alcohols, such as methanol, ethanol, isopropanol, butanol or glycol, for example, and also their ethers and esters, ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone or cyclohexanone, for example, strongly polar solvents, such as dimethyl sulphoxide, polyether-modified trisiloxanes and water.

All suitable and compatible carriers may in principle be used. Suitable carriers are in particular: for example, ammonium salts and ground natural minerals such as kaolins, clays, talc, chalk, quartz, attapulgite, montmorillonite or diatomaceous earth, and ground synthetic minerals, such as finely divided silica, alumina and natural or synthetic silicates, resins, waxes and/or solid fertilizers. Mixtures of such carriers may likewise be used. Carriers suitable for granules include the following: for example, crushed and fractionated

natural minerals such as calcite, marble, pumice, sepiolite, dolomite, and also synthetic granules of inorganic and organic meals, and also granules of organic material such as sawdust, paper, coconut shells, maize cobs and tobacco stalks; or also compounds like sugars.

- 5 Liquefied gaseous extenders or solvents may also be used. Particularly suitable are those extenders or carriers which at standard temperature and under standard pressure are gaseous, examples being aerosol propellants, such as halogenated hydrocarbons, and also butane, propane, nitrogen and carbon dioxide.

10 Examples of emulsifiers and/or foam-formers, dispersants or wetting agents having ionic or nonionic properties, or mixtures of these surface-active substances, are salts of polyacrylic acid, salts of lignosulphonic acid, salts of phenolsulphonic acid or naphthalenesulphonic acid, polycondensates of ethylene oxide with fatty alcohols or with fatty acids or with fatty amines, with substituted phenols (preferably alkylphenols or arylphenols), salts of sulphosuccinic esters, taurine derivatives (preferably alkyltaurates), phosphoric esters of polyethoxylated alcohols or phenols, fatty acid esters of polyols, and  
15 derivatives of the compounds containing sulphates, sulphonates and phosphates, examples being alkylaryl polyglycol ethers, alkylsulphonates, alkyl sulphates, arylsulphonates, protein hydrolysates, lignin-sulphite waste liquors and methylcellulose. The presence of a surface-active substance is advantageous if one of the active compounds and/or one of the inert carriers is not soluble in water and if application takes place in water. Preferred emulsifiers are alkylaryl polyglycol ethers.

20 Further auxiliaries that may be present in the formulations and in the application forms derived from them include colorants such as inorganic pigments, examples being iron oxide, titanium oxide, Prussian Blue, and organic dyes, such as alizarin dyes, azo dyes and metal phthalocyanine dyes, and nutrients and trace nutrients, such as salts of iron, manganese, boron, copper, cobalt, molybdenum and zinc.

25 Stabilizers, such as low-temperature stabilizers, preservatives, antioxidants, light stabilizers or other agents which improve chemical and/or physical stability may also be present. Additionally present may be foam-formers or defoamers.

30 Furthermore, the formulations and application forms derived from them may also comprise, as additional auxiliaries, stickers such as carboxymethylcellulose, natural and synthetic polymers in powder, granule or latex form, such as gum arabic, polyvinyl alcohol, polyvinyl acetate, and also natural phospholipids, such as cephalins and lecithins, and synthetic phospholipids. Further possible auxiliaries include mineral and vegetable oils.

There may possibly be further auxiliaries present in the formulations and the application forms derived from them. Examples of such additives include fragrances, protective colloids, binders, adhesives, thickeners, thixotropic substances, penetrants, retention promoters, stabilizers, sequestrants, complexing agents, humectants and spreaders. Generally speaking, the active compounds may be combined with any solid or liquid additive commonly used for formulation purposes.

Suitable retention promoters include all those substances which reduce the dynamic surface tension, such as dioctyl sulphosuccinate, or increase the viscoelasticity, such as hydroxypropylguar polymers, for example.

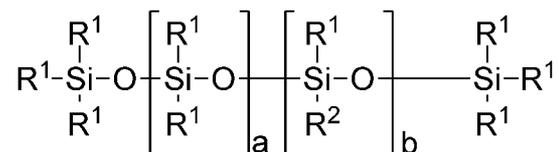
Suitable penetrants in the present context include all those substances which are typically used in order to enhance the penetration of active agrochemical compounds into plants. Penetrants in this context are defined in that, from the (generally aqueous) application liquor and/or from the spray coating, they are able to penetrate the cuticle of the plant and thereby increase the mobility of the active compounds in the cuticle. This property can be determined using the method described in the literature (Baur *et al.*, 1997, Pesticide Science 51, 131-152). Examples include alcohol alkoxyates such as coconut fatty ethoxylate (10) or isotridecyl ethoxylate (12), fatty acid esters such as rapeseed or soybean oil methyl esters, fatty amine alkoxyates such as tallowamine ethoxylate (15), or ammonium and/or phosphonium salts such as ammonium sulphate or diammonium hydrogen phosphate, for example.

The composition comprising *P. lilacinum* or spores thereof preferably comprises between 0.00000001% and 98% by weight of active compound or, with particular preference, between 0.01% and 95% by weight of active compound, more preferably between 0.5% and 90% by weight of active compound, based on the weight of the formulation.

The composition to be applied in connection with the present invention may comprise compatible adjuvants such as IMBIREX<sup>®</sup>, BU EXP<sup>®</sup> 1136, AFINITY<sup>®</sup>, LI 700 PCP<sup>®</sup> 230026, IRRIG AID GOLD<sup>®</sup>, PENEAL<sup>®</sup>, NEOWETT<sup>®</sup>, QUADRA TECK<sup>®</sup>, RESPOND<sup>®</sup> 3, TRIFOLIO S FORTE<sup>®</sup>, SILWET COPOLYMER<sup>®</sup> 480, SILWET GOLD<sup>®</sup>, TWEEN<sup>®</sup> 20, BREAK-THRU<sup>®</sup> 240 (0.05%), BREAK-THRU<sup>®</sup> 240 (0.2%), SILWET<sup>®</sup> L-77 COPOLYMER and BIOLINK<sup>®</sup>.

In a preferred embodiment, said composition is a liquid composition and further comprises at least 75% polyether-modified trisiloxane. Such formulations are disclosed e.g., in WO 2012/163322. In the course of the present invention, it has surprisingly been found that a formulation comprising at least 75% polyether-modified trisiloxane has an even bigger effect on certain plant growth or plant health properties, see Examples 3 and 4.

In a more preferred embodiment, said polyether-modified trisiloxane is of formula I



Formula (I)

5

where

R<sup>1</sup> represents independent from each other identical or different hydrocarbyl radicals having 1-8 carbon atoms, preferred methyl-, ethyl-, propyl- and phenyl radicals, particularly preferred are methyl radicals.

a = 0 to 1, preferred 0 to 0.5, particularly preferred 0,

10 b = 0.8 to 2, preferred 1 to 1.2, particularly preferred 1,

in which: a + b < 4 and b > a, preferred a + b < 3 and particularly preferred a + b < 2.

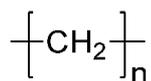
R<sup>2</sup> represents independent from each other identical or different polyether radicals of general formula (II)

15  $\text{-R}^3\text{O}[\text{CH}_2\text{CH}_2\text{O}]_c[\text{CH}_2\text{CH}(\text{CH}_3)\text{O}]_d[\text{CHR}^4\text{CHR}^4\text{O}]_e\text{R}^5$

Formula (II)

R<sup>3</sup> = independent from each other identical or different, bivalent hydrocarbyl radicals having 2 - 8 carbon atoms, which are optionally interrupted by oxygen atoms, preferred rest is the general formula (III) where n = 2 - 8,

20 particularly preferred  $\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-}$ ,



Formula (III)

25 R<sup>4</sup> = independent from each other identical or different hydrocarbyl radicals having 1-12 carbon atoms or hydrogen radical, preferably a methyl-, ethyl-, phenyl- or a hydrogen radical.

R<sup>5</sup> = independent from each other identical or different hydrocarbyl radicals having 1-16 carbon atoms, which are optionally contain urethane functions, carbonyl functions or carboxylic acid ester functions, or hydrogen radical, preferred methyl or H, particularly preferred H,

30

C = 0 to 40, preferred 1 to 15, particularly preferred 2 to 10

d = 0 to 40, preferred 0 to 10, particularly preferred 1 to 5

e = 0 to 10, preferred 0 to 5, particularly preferred 0,

in which  $c + d + e > 3$

- 5 The polyether-modified trisiloxanes described above can be prepared by methods well known to the practitioner by hydrosilylation reaction of a Si-H containing siloxane and unsaturated polyoxyalkylene derivatives, such as an allyl derivative, in the presence of a platinum catalyst. The reaction and the catalysts employed have been described for example, by W. Noll in "Chemie und Technology der Silicone", 2<sup>nd</sup> ed., Verlag Chemie, Weinheim (1968), by B. Marciniak in "Appl. Homogeneous Catal. Organomet. Compd. 1996, 1, 487). It is common knowledge that the
- 10 hydrosilylation products of SiH-containing siloxanes with unsaturated polyoxyalkylene derivatives can contain excess unsaturated polyoxyalkylene derivative.

Examples of water soluble or self-emulsifiable polyether-modified (PE/PP or block-CoPo PEPP) trisiloxanes include but are not limited to those described by CAS-No. 27306-78-1 (e.g., SILWET<sup>®</sup> L77

15 from MOMENTIVE), CAS-No. 134180-76-0 (e.g., BREAK-THRU<sup>®</sup> S233 or BREAK-THRU<sup>®</sup> S240 from Evonik), CAS-No 67674-67-3 (e.g., SILWET<sup>®</sup> 408 from WACKER), other BREAK-THRU<sup>®</sup>-types, and other SILWET<sup>®</sup>-types.

Preferred polyether-modified trisiloxanes include those described by CAS-No. 134180-76-0, in particular

20 BREAK-THRU<sup>®</sup> S240.

A formulation according to the invention comprising a polyether-modified trisiloxane, in addition to the advantages described above reduce surface tension even in high dilutions, e.g., for soil applications, since such formulation contains a high concentration of polyether-modified trisiloxane being a surfactant. This

25 may promote the advantageous PGP or plant health promoting properties of *P. lilacinum*.

In a more preferred embodiment said polyether-modified trisiloxane is BREAK-THRU<sup>®</sup> S240.

In another preferred embodiment, said composition further comprises up to 9% fumed silica. Exemplary

30 percentages of fumed silica range between 1 wt.-% and 9 wt.-%, such as 2 wt.-%, 3 wt.-%, 4 wt.-%, 5 wt.-%, 6 wt.-%, 7 wt.-% and 8 wt.-% and any value in between.

Fumed silica, also known as pyrogenic silica, either hydrophilic or hydrophobic, usually is composed of amorphous silica fused into branched, chainlike, three-dimensional secondary particles which then

35 agglomerate into tertiary particles. The resulting powder has an extremely low bulk density and high surface area. Both hydrophilic and hydrophobic fumed silica can be used in the present invention.

Fumed silica usually has a very strong thickening effect. The primary particle size is ca. 5–50 nm. The particles are non-porous and have a surface area of ca. 50–600 m<sup>2</sup>/g.

5 Hydrophilic fumed silica is made from flame pyrolysis of silicon tetrachloride or from quartz sand vaporized in a 3000°C electric arc. Major global producers are Evonik Industries, tradename AEROSIL®; Cabot Corporation, tradename CAB-O-SIL®; Wacker Chemie, HDK product range; and OCI, tradename KONASIL®.

10 Hydrophilic fumed silica can be hydrophobized by further treatment with reactive silicium-containing agents in order to modify the physicochemical properties of the silica. Typically hydrophobisation takes place by treatment of a hydrophilic fumed silica with agents like hexaalkyldisilanes (e.g., ((CH<sub>3</sub>)<sub>3</sub>Si)<sub>2</sub>), trialkylsilylchlorides (e.g., (CH<sub>3</sub>)<sub>3</sub>SiCl) or dialkyldichlorsilanes (e.g., (CH<sub>3</sub>)<sub>2</sub>SiCl<sub>2</sub>). Hydrophobized fumed silica is available e.g., from Evonik Industries (AEROSIL® R-types), and Cabot (CAB-O-SIL®).

15 Best results are obtained using a hydrophilic fumed silica having a BET surface area of 150 to 350 m<sup>2</sup>/g, e.g., 150, 200, 250, 300 or 350.

In a more preferred embodiment said fumed silica is AEROSIL®.

20 In connection with the present invention, the composition described above comprising spores of *P. lilacinum*, polyether-modified trisiloxane and fumed silica and only traces of other ingredients performs even better than a WG formulation. Such compositions are disclosed in WO2016/050726 which is incorporated herein by reference.

25 For the avoidance of doubt, all compositions used in the present invention and comprising *P. lilacinum* were shown to have the claimed effect. However, the above liquid formulation showed an even bigger effect.

30 The final dosage of infective propagules of *Purpureocillium lilacinum* strain 251 is normally in the order of between about 1 x 10<sup>4</sup> and about 1 x 10<sup>8</sup>, preferably between about 1 x 10<sup>5</sup> and about 2 x 10<sup>7</sup>, more preferably between 1 x 10<sup>5</sup> and 5 x 10<sup>6</sup>, such as 2 x 10<sup>5</sup>, 5 x 10<sup>5</sup>, 1 x 10<sup>6</sup> or 2 x 10<sup>6</sup> spores per gram of soil for nursery applications and for field applications.

35 The present invention furthermore relates to the use of a composition comprising the fungus *Purpureocillium lilacinum* or spores thereof for promoting or improving plant health or plant growth promotion.

All embodiments as described for the method of the invention may as well be applied in the use of the present invention.

5 The figures show:

FIG. 1A: Nematicidal activity of *Purpureocillium lilacinum* strain 251 and fluopyram against *Meloidogyne incongita*. Mean of 3 replicates + standard error (SE).

10 FIG. 1B: Plant health activity of *Purpureocillium lilacinum* strain 251 and fluopyram in presence of *Meloidogyne incongita*. Mean of 3 replicates + standard error (SE). Significant differences ( $p \leq 0.05$ ) are indicated by asterisks.

FIG. 2A: Total root surface area of tomato treated with a liquid formulation of *P. lilacinum* strain 251 (BIOACT<sup>®</sup> liquid); 4 reps per treatment. Run unpaired "T Test," confidence level of 95%, examining  
15 threshold of significance,  $p \leq 0.05$ .

FIG. 2B: Whole plant biomass of tomato treated with a liquid formulation of *P. lilacinum* strain 251 (BIOACT<sup>®</sup> liquid); Statistically significant with a p-value of 0.0249, t value of 2.973 and df=6 degrees of  
20 freedom).

FIG. 2C: Nematode efficacy showing average number of egg masses. Statistically significant with a  $P < 0.0001$ , t value of 13.36, df=6.

25 FIG. 3A: Whole plant biomass of tomato treated with different formulations of *P. lilacinum* strain 2514 reps per treatment; One-Way analysis of variance (ANOVA).

FIGS. 3B, 3C, 3D: Average root weight, total root surface area (cm<sup>2</sup>) (p-value of 0.0270) and *M. javanica* egg masses (p-value of 0.0007) for the treatments shown in 3A.  
30

FIG. 4: Leaf surface area of tomatoes treated with formulations of *P. lilacinum* strain 251 as compared to other fungal strains.

FIG. 5A: Fresh whole plant biomass of corn plants treated with a liquid formulation of *P. lilacinum* strain  
35 251; \* $p = 0.05$  or less; \*\* $p = 0.01$  or less.

FIG. 5B and 5C: Root length (%B) and total number of tip, forks and crossings (TFC) of the trials shown in FIG. 5A.

5 FIG. 6: Trial tomato treatment with a liquid formulation of *P. lilacinum* strain 251 in 80% sand and 20% Sunshine #3. A: Gall rating; B: Egg count; C: dry shoot weight; D: dry root weight; E: total vegetative weight.

10 FIG. 7: Trial tomato treatment with a liquid formulation of *P. lilacinum* strain 251 in 80% Profile and 20% Sunshine #3. A: Gall rating; B: total fruit weight; C: dry shoot weight; D: total vegetative weight.

FIG. 8: Trial cucumber treatment with a liquid formulation of *P. lilacinum* strain 251 in 80% sand and 20% Sunshine #3. A: Gall rating; B: Egg count; C: dry root weight.

15 FIG. 9: Total fruit weight from trial tomato treatment with a liquid formulation of *P. lilacinum* strain 251 in 80% Profile and 20% Sunshine #3.

FIG. 10: Efficacy (bars) and yield (dots) of trials with PL251 in cucumber (10A) and tomato (10B).

20 FIG.11: Total root length (cm, Fig. 11A) and root surface area (cm<sup>2</sup>, Fig. 11B) of tomato plantlets treated with spores of three *P. lilacinum* strains in comparison with untreated control plantlets.

The examples further illustrate the invention in a non-limiting fashion.

**Example 1: Drench application of *Purpureocillium lilacinum* strain 251 (PL 251) in tomato**

To prepare a suitable dosage form the formulated product PL251 (liquid formulation of *P. lilacinum* comprising BREAK-THRU® S240 and AEROSIL®) is diluted with water to the desired concentration.

A quantity of 5,000 cm<sup>3</sup> of sandy loam soil, pH 6.8 per pot is mixed with 150,000 infective units (mixed population of eggs and juveniles) of the Southern Root Knot Nematode (*Meloidogyne incognita*). The desired concentration of *Purpureocillium lilacinum* strain 251 is drenched in 400 mL of solution (application A) to obtain 80% field capacity. Pots are incubated at 25°C until transplanting of tomato seedlings (*Lycopersicon lycopersicum*) at 7 days after drench application A. At the day of transplanting a second drench application (application B) is carried out with 400 mL of solution.

After transplanting of tomato seedlings several post-plant application patterns are tested using 400 mL of solution per application (Table A).

In addition, 10 mg of the nematicide fluopyram (VELUM® PRIME, suspension concentrate SC 400) is applied at transplanting and used as a chemical reference. The non-treated control (UTC) is drenched with 400 mL of water at each application. The experiment is kept for 7 weeks after transplanting at 25°C.

**Table A: *Purpureocillium lilacinum* strain 251 drench test in tomato**

Application	A	B	C	D
Application timing	7 d prior to transplanting	at transplanting	4 weeks after transplanting	6 weeks after transplanting
Treatment				
UTC	water	water	water	water
1	5.5 x 10 <sup>5</sup> viable spores per cm <sup>3</sup> substrate	5.5 x 10 <sup>5</sup> viable spores per cm <sup>3</sup> substrate	5.5 x 10 <sup>5</sup> viable spores per cm <sup>3</sup> substrate	water
2	5.5 x 10 <sup>5</sup> viable spores per cm <sup>3</sup> substrate	5.5 x 10 <sup>5</sup> viable spores per cm <sup>3</sup> substrate	water	5.5 x 10 <sup>5</sup> viable spores per cm <sup>3</sup> substrate
3	5.5 x 10 <sup>5</sup> viable spores per cm <sup>3</sup> substrate	5.5 x 10 <sup>5</sup> viable spores per cm <sup>3</sup> substrate	5.5 x 10 <sup>5</sup> viable spores per cm <sup>3</sup> substrate	5.5 x 10 <sup>5</sup> viable spores per cm <sup>3</sup> substrate
4	water	10 mg fluopyram / plant	water	water

After the specified period the nematicidal activity is determined on the basis of the percentage of gall reduction. 100 % means that no galls were found; 0% means that the number of galls found on the roots of treated plants was equal to that in untreated control plants. Moreover the shoot biomass is also determined to assess the overall plant health status.

The experimental set up is fully randomized and comprised three replicates per treatment. One-way analysis of variance (ANOVA) is carried out for shoot biomass using a threshold for significance of  $p \leq 0.05$  and a Bonferroni posttest to compare all treatments against the UTC.

10 The bionematocide PL251 shows not only weak nematicidal activity at a rate of  $5.5 \times 10^5$  spores per  $\text{cm}^3$  of substrate and at the used nematode pressure of 3,000 *Meloidogyne incognita* per 100  $\text{cm}^3$  of soil. This finding is independent of the application patterns tested in this experiment (FIG. 1A). The chemical nematicide fluopyram shows excellent biocontrol activity at 10 mg per plant.

15 The lacking performance of PL251 is likely because of the high nematode pressure used in the present study. Kiewnick et al. 2011<sup>1</sup> reported approx. 50% biocontrol efficacy at 400 infective units of *Meloidogyne incognita* per 100  $\text{cm}^3$  of soil. At a higher nematode density of 1,600 infective units, only 22 % biocontrol were reported. However, the nematode pressure used in the present study (3,000 infective units per 100  $\text{cm}^3$  soil) was considerably higher compared to that of Kiewnick *et al.* 2011.

20 Despite its weak nematicidal performance PL251 improves tomato shoot fresh weight by 12-26% depending on the post-plant application pattern.

Statistical analysis reveals a significant improvement of shoot fresh weight after nematicide treatment and a highly significant shoot fresh weight increase following application of PL251 at ABC (ANOVA,  $p=0.0222$ ,  $df=14$ ; Bonferroni multiple comparison test,  $t=4.191$  for UTC vs. PL251 at ABC). The results clearly show that PL251 displays an additional plant health effect independent of its nematicidal potential leading to improved shoot weight in tomato.

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<sup>1</sup> Kiewnick, S.; Neumann, S.; Sikora, R.A.; Frey, J.E. 2011: Effect of *Meloidogyne incognita* Inoculum Density and Application Rate of *Paecilomyces lilacinus* Strain 251 on Biocontrol Efficacy and Colonization of Egg Masses Analyzed by Real-Time Quantitative PCR. **Phytopathology**, Vol. 101, No. 1, 2011

**Example 2: Growth Chamber Tomato In-planta Jar assay to evaluate the efficacy of BIOACT® against root-knot-nematodes as well as its plant growth promotion (PGP) effect**

Treatments

- 5 1. UTC-75 mLs of water
2. BIOACT® applied at planting 1 µL (1.08mg) per 100 mL of soil,  $5.5 \times 10^5$  viable spores per cm<sup>3</sup> substrate

\*Soil Combination added 225 mL to the plastic jar

10

Procedure: Eight 300 mL (10oz) polypropylene flip top jars for this assay were prepared to examine fungal colonization of a liquid formulation of *P. lilacinum* strain 251, PGP effects, and to determine nematode reduction. Each jar was filled with soil combination (type of soil to mimic field soil conditions). Planted tomato seeds, ACE 55 Tomato Variety, Mountain Valley Seed Co. This assay is to examine  
15 *Purpureocillium lilacinum* fungal growth in-planta, determining a soil type that would provide a carbon source for the fungus to grow, colonize the soil and protect the roots from root-knot nematode infection.

The treatment jars received 75 mL of drench solution. Each UTC jar was watered with 75 mL of tap water at time of planting. Each treatment was placed in sterile greenhouse flats to eliminate cross-contamination. All treatments were placed in a plant growth chamber. Experiment duration was 7 week trial, the settings  
20 for the growth chamber were set for photoperiod of 12hrs of light and 12hrs of dark, light intensity 320 µMol, temperature of 25°C for light period, 20°C for dark period.

Tomato In-planta jar assay takedown: Each root system was washed from the 225 mLs of soil in a plastic 3 quart pitcher. As roots were cleaned, they were placed on paper towels to dry excess water running off.

Analysis of Tomato Roots: Tomato roots were analyzed using the program WinRhizo, Regent Instruments,  
25 Inc. (Arsenault et al, 1995). This program provides for a complete plant root measurement and analysis. WinRhizo allows looking at the length, area, volume, topology, and architecture of the plant roots. Each UTC and Treatment were scanned to examine the total root surface area (cm<sup>2</sup>).

Staining RKN egg masses: Once roots were scanned using WinRhizo, the roots were stained with Erioglaurine 1 mg/L solution for 15 mins. Each root system was submerged in the solution. The  
30 Erioglaurine Blue Solution stains the egg masses in bright blue (making it easier to visualize and count the fully developed females). The stain sticks to the gelatinous matrix that surrounds the RKN egg masses from the posterior end of the female nematode.

**Results:**

Total Root Surface Area: Comparing untreated with BIOACT<sup>®</sup> DC drench, on average an increase in root surface area is visible in the treatment. The treatment average measurement was 19.916 cm<sup>2</sup>, untreated average was 18.669 cm<sup>2</sup>. (see FIG. 2A)

Whole Plant Biomass: Total fresh shoot (whole plant biomass) of Untreated compared to BIOACT<sup>®</sup> Treatment was evaluated. BIOACT<sup>®</sup> treatment had significantly higher fresh whole plant biomass than UTC. Larger tomato roots treated with BIOACT<sup>®</sup> drench showed an increase in lateral root growth as compared to UTC (see FIG. 2B).

Nematode efficacy showing average number of egg masses: Nematicidal activity of *Purpureocillium lilacinum* strain 251 against *Meloidogyne javanica*. Overall, there was a decrease in the number of egg masses developed on BIOACT<sup>®</sup> treatment compared to UTC. Effects of BIOACT<sup>®</sup> treatment were significantly different from the UTC. One application of BIOACT<sup>®</sup> treatment showed a significant reduction of *M. javanica* egg masses. The average number of egg masses in the UTC was 161 compared to that in the BIOACT<sup>®</sup> treatment of 65.25 (see FIG. 2C). Evaluation of the number of egg masses definitely illustrates a smaller number of J2s penetrating and fully developing into a female adult, decreasing the number of egg mass production.

**Example 3: Growth Chamber Tomato In-planta Jar assay to evaluate the efficacy of two different formulations of BIOACT® (liquid and WG) against root-knot nematodes and to compare their PGP performance**

5 Treatments

3. UTC-75 mLs of water
4. BIOACT® applied at planting  $5.5 \times 10^5$  viable spores per  $\text{cm}^3$  substrate
5. BIOACT® WG applied at planting 5.0 mg per 100 mL of soil (corresponding to  $1.75 \times 10^6$  viable spores per 100 mL of soil
- 10 6. Blank Formulation applied at planting 1.08 mg per 100 mL of soil

\*Soil Combination added 225 mL to the plastic jar

Procedure: Twenty 300 mL (10oz) polypropylene flip top jars were prepared to examine BIOACT® fungal colonization, PGP effects, and to determine nematode reduction. Each jar was filled with soil combination  
15 (type of soil to mimic field soil conditions). Planted tomato seeds were from the variety ACE 55, Mountain Valley Seed Co.

The treatment jars received 75 mLs of drench solution. Each UTC jar was watered with 75 mLs of tap water at time of planting. The blank formulation comprised the formulants of the liquid formulation, i.e.,  
20 BREAK-THRU® S240 and AEROSIL®, each jar received the same amount as the one treated with the liquid formulation of BIOACT®, 2.7 mg in 75 mLs of water. Pots treated with BIOACT® WG (wetttable granule received 125 mg in 75 mLs of water per treatment. Each treatment was placed in sterile greenhouse flats to eliminate cross-contamination. All treatments were placed in a plant growth chamber. The experiment duration was 7 weeks, the settings for the growth chamber were set for photoperiod of 12hrs of light and  
25 12hrs of dark, light intensity  $320 \mu\text{Mol}$ , temperature of  $25^\circ\text{C}$  for light period,  $20^\circ\text{C}$  for dark period.

Two weeks after planting tomato seeds, inoculated each jar with 300 active infective stage juveniles of *Meloidogyne javanica* (freshly hatched J2s from our root knot nematode tomato cultures).

30 Tomato In-planta jar assay takedown: Each root system was washed from the 225 mLs of soil in a plastic 3 quart pitcher. As roots were cleaned, they were placed on paper towels to dry excess water running off. Each tomato plant was weighed to determine the total plant biomass. Afterwards, the shoots were cut off and discarded. Each root system was weighed per treatment to determine the weight of treatment.

Staining RKN egg masses: Roots were stained with Erioglaurine 1 mg/L solution for 15 mins. Each root system was submerged in the solution.

Statistical Analysis: Experiment set up is randomized and comprises 4 reps per treatment, One-Way  
5 analysis of variance (ANOVA) was performed.

### **Results:**

There is a significant increase in whole plant biomass in BIOACT® liquid treatment compared to UTC and  
10 blank. BIOACT® WG also performed better than Blank and untreated (see FIG. 3A).

The weight of root mass was examined for each treatment. There is a significant increase in root weight of  
BIOACT® liquid compared to UTC and Blank. It is evident that PGP present with BIOACT® compared to  
Blank, and UTC (see FIG. 3B).

The total root surface area (cm<sup>2</sup>) of each treatment was examined using the WhinRhizo root analysis  
15 program. BIOACT® treatment results in significantly more average root weight as compared to UTC and  
Blank (see FIG. 3C).

The number of RKN egg masses were counted for each treatment (4 reps per treatment). There is  
significance in the reduction of the number of egg masses in BIOACT® liquid treatment compared to UTC  
and Blank treatments.

**Example 4: Tomato Drench Assay to evaluate PGP properties of *P. lilacinum* strain 251 as compared to other fungal strains**

5 Assay: 30 day evaluation to allow tomatoes to grow and examine the foliar canopy, determination of increase in leaf surface area.

Treatment List:

#	TREATMENT	RATES
1	Untreated Control	Sterile DI Water
2	Blank Formulation (formulants of liquid formulation)	1.08 mg/100 mL of soil
3	<i>Purpureocillium lilacinum</i> strain 251	1.7 x 10 <sup>8</sup> spores per mL
4	BIOACT <sup>®</sup> liquid 1x	1.08 mg/100 mL of soil 5.5 x 10 <sup>5</sup> viable spores per cm <sup>3</sup> substrate
5	BIOACT <sup>®</sup> liquid 10x	10.8 mg/100 mL of soil 5.5 x 10 <sup>6</sup> viable spores per cm <sup>3</sup>
6	<i>Penicillium bilaii</i>	1.11 x 10 <sup>9</sup> spores per mL
7	<i>Trichoderma virens</i>	1.27 x 10 <sup>8</sup> spores per mL

10 Protocol: 6 x 7 plug trays were cut from a 10 x 20 (200) cell plug tray (Hummert, Int.) Each cell tray was filled with potting soil and every other cell was seeded with one tomato seed (Washington Cherry Tomato Variety). Each seeded cell in the tray was treated with 2 mL of material using a serological pipette. Plug trays were watered from the bottom by flooding a clam shell tray. Flats were then placed under high intensity lights (~300 Einsteins, set to 16 hour light/8 hour dark schedule) and watered once a day. Samples were compared based on volume. Every 2-3 days, clam shells were randomized. Plants were rated 30 days after planting. Three replicates per treatment were prepared.

Drench Solution Preparation: Fungal strains (*Penicillium*, *Trichoderma* and *P. lilacinum*) were streaked onto Potato Dextrose Agar plates to enable fungal spores to grow.

- 20
1. Set up day: 10 mL of sterile DI water was poured on fungal plate.
  2. Fungal spores were scrapped with an L-shaped rod to break away the spores from the agar, prepare spore suspension.
  3. The spore suspension was collected in a falcon tube, and then passed through a sterile piece of cheesecloth to ensure only spores (no agar) were present.

4. Spore suspension was quantified using a hemocytometer and then diluted to the desired concentrations.
5. 2 mL of spore suspension was added to each seeded cell in a tray.

5 Measurements: Leaf surface area was examined using Image J software and documented by taking top view images of each treatment rep block using a Nikon Camera and tripod. In each picture a ruler placed next to each tomato tray treatment which is used as reference to calibrate the software's scale.

### **Results**

10 **Leaf Surface Area (see FIG. 4):** The following strains and rates had significant increases in leaf surface area compared to the "Untreated Water Control" trays: *Trichoderma virens*, *Purpureocillium lilacinum*, and both rates of BIOACT® liquid (Standard and 10x). Overall, BIOACT® liquid 1x and 10x as well as the unformulated *P. lilacinum* strain 251 had a larger increase in leaf surface foliar canopy compared to UTC, and *P. bilaii*.

15 BIOACT® liquid 1x and BIOACT® liquid 10x have a larger leaf surface area compared to all other treatments. Examining BIOACT® liquid treatments, the surfactant in the formulation could be aiding in the movement of *Purpureocillium lilacinum* strain 251 spores in the soil mix.

20 **Example 5: Corn Drench Assay with a liquid formulation of spores of *P. lilacinum* strain 251 (BIOACT® liquid)**

Different doses of a liquid formulation of *Purpureocillium lilacinum* strain 251 (BIOACT® liquid) were tested in a small pot drench assay on corn to evaluate plant growth promotion.

25 Start Date: 8/19/15: Set up experiment, 9/02/15: Take down experiment (14 days experiment).  
Method: 3 x 3 x 3 cubic inch small pots were filled with autoclaved 3:1 Soil:Sand mix. There were 4 pots per treatment replicate (12 pots total per treatment). 2 sterilized GP7169 GT corn seeds were planted 1-inch deep in each pot, 2 inches apart. 50 milliliters of drench solution were applied per pot at the rate specified  
30 for each treatment (see "Calculations for Drench" section).

Environment: Light Racks

- Light Temperature Range = 20°C low to 30°C high
- Relative Humidity: low 21% to high 62%

**Treatments:**

1. UTC
2. VOTIVO® 5% (Assay Positive Control)
3. BIOACT® 2.13 x 10<sup>8</sup> spores/pot
- 5 4. BIOACT® 2.13 x 10<sup>9</sup> spores/pot

**Calculations for Drench:** All BIOACT® drench solutions were made at 600 mL per treatment.

For BIOACT® DC

- 10 1. Standard rate: 1.08 mg/100 mL soil x 4.25 = 4.59 mg/pot 2.13 x 10<sup>8</sup> spores/pot
2. 10x rate: 10.8 mg/100 mL soil x 4.25 = 45.9 mg/pot 2.13x10<sup>9</sup> spores/pot
3. For VOTIVO® 5%, 35 mL of VOTIVO® were added into 665 mL of DI water, equivalent to 9.15 x 10<sup>7</sup> CFU/pot.

15 **Observations Notes and Measurements:**

1. Germination (poor, normal), Phytotoxicity (if any)
2. Whole Plant Biomass (fresh)
3. WinRhizo Root Analysis

**Results:**

20 Germination: normal. Phytotoxicity: none observed

Fresh Whole Plant Biomass (FWPB) (FIG. 5A): Up to 24 plants were measured. Plants that were not measured include non-germinated seedlings and incomplete plants (incomplete plants are ones without roots or shoots). A dose response was observed with the BIOACT® treatments with the highest rate (BIOACT® 10x) having the best PGE (plant growth enhancement) (according to t-test analysis, if p<0.1, then there is a significant difference). The difference between the highest rate and standard rate was 15% although the standard rate of BIOACT® was also significantly better UTC.

30 WinRHIZO analysis of 6 roots per treatment (sub-sample of up to 24 possible roots). Results are shown in FIG. 5B and 5C. Overall, BIOACT® liquid 1x and BIOACT® liquid 10x treatments had higher values of RL and total number of TFC.

BIOACT® dose rates were tested in the small pots drench assay for plant growth enhancement. BIOACT® doses were tested at standard rate (4.59 mg or 2.13 x 10<sup>8</sup> spores/pot) and 10 times the standard rate (45.9 mg

or  $2.13 \times 10^9$  spores /pot) alongside UTC (negative control), and VOTIVO<sup>®</sup> 5% (positive control). Fresh whole plant biomass (FWPB) was taken 14 days after planting. A dose response with BIOACT<sup>®</sup> was observed in the assay. BIOACT<sup>®</sup> standard rate and 10x rate showed significantly higher FWPB than UTC. 6 roots of each treatment were analyzed using the WinRHIZO. A dose rate was observed and similarly to the FWPB results, BIOACT<sup>®</sup> standard and 10x rate had the best root length(RL), and total number of tips(TFC), forks, and crossings. VOTIVO<sup>®</sup> 5% roots generally did not show better root architecture than UTC. In conclusion, plant growth enhancement with BIOACT<sup>®</sup> was shown in a 14-day corn drench bioassay.

10 **Example 6: Nematode and greenhouse trials to identify differences in PGP (plant growth promotion) and nematode galling in plants treated with a liquid formulation of *P. lilacinus* strain 251 (BIOACT<sup>®</sup>) in comparison with the UTC in two different substrates**

15 **General Materials and Methods:**

Tomatoes:

Variety: Ace 55

Tomatoes were planted into 200 cell plug trays. The seeds were distributed in a checkerboard pattern to allow sufficient spacing for the plants to expand prior to transplanting. The tomatoes were transplanted into 5.5" pots after two weeks.

Cucumbers:

Variety: Sultan

The cucumbers were direct seeded into 32 oz. cups.

Potting mixes:

Sand: Coarse Play Sand

Potting soil

30 Kaolin clay

The plants were grown in two different potting mixes either comprising sand and soil or a clay-based additive and soil.

35

**Experimental Design:**Treatments:

- 5
1. UTUI-Untreated uninfested
  2. UTC-Untreated Control, infested with RKN (root knot nematodes)
  3. BIOACT® 1x - One application of BIOACT®, infested with RKN nematodes
  4. BIOACT® 2x - Two applications of BIOACT®, infested with RKN nematodes

Replicate Number: 10

10

BIOACT® Drench Applications: Prepared BIOACT® drench solutions for each 4 trials, each plant received 30 mLs of drench solution. The spore load per application was  $5.5 \times 10^5$  viable spores per  $\text{cm}^3$  substrate.

Pesticide Applications: Cucumbers received fungicide treatment in addition

15

**Tomato trial in sandy soil soil**

Plant Date:	7/11/2016
Transplant Date:	7/25/2016
Infestation Date:	8/9/2016
1st Treatment Date:	7/29/2016
2nd Treatment Date:	8/16/2016
Take Down:	10/3/2016

- 20 **Results:** There was a significant difference in dry shoot weight, dry root weight and total vegetative weight between the UTC and the BIOACT® treatments (FIGS. 6C, 6D and 6E). Also gall rating and egg count were significantly different (FIGS. 6A and 6B).

**Tomato trial in soil mixed with clay soil**

25

Plant Date:	7/18/2016
Transplant Date:	8/1/2016
Infestation Date:	8/17/2016
1st Treatment Date:	8/9/2016

2nd Treatment Date: 8/23/2016  
Take Down: 9/30/2016

**Results:** Significant reduction in gall development visible in BIOACT® treatments compared to control (UTC) (FIG. 7A), there was good nematode infection (galling) seen in the UTC. Differences in total fruit weight (FIG. 7B) were also observed between UTUI and treated plants. There was a significant difference in dry shoot weight between the UTC and the BIOACT® 2x (FIG. 7C) and total vegetative weight (FIG. 7D).

#### Cucumber trial in sandy soil

10

Plant Date: 7/18/2016  
Infestation Date: 8/9/2016  
1st Treatment Date: 7/29/2016  
2nd Treatment Date: 8/16/2016  
Take Down: 10/3/2016

**Results:** Significant differences were observed between BIOACT® treatments and UTC (with nematodes) in gall rating and egg count (FIGS. 8A and B). The reduction in the number of eggs compared to UTC was visible. Differences were also observed in dry root weight (FIG. 8C).

15

#### Cucumber trial in soil mixed with clay

Plant Date: 7/25/2016  
Infestation Date: 8/17/2016  
1st Treatment Date: 8/9/2016  
2nd Treatment Date: 8/23/2016  
Take Down: 10/3/2016

**Results:** BIOACT® liquid 1x performed better than the other three treatments. There was significant increase in fruit yield with one application of BIOACT® DC compared to UTUI and UTC. Significant differences in the total fruit weight were observed (FIG. 9A).

20

**Example 7: Trials to evaluate nematicidal efficacy and yield increase by *P. lilacinum* strain 251 in cucumber and tomato in greenhouse area**

**General Materials and Methods:**

5 This yield study was carried out in high commercial greenhouses representative for most important cucumber and tomato areas in Spain and Italy, respectively. In total 6 trials were conducted in following provinces and trial sites:

**Yield program for cucumber:**

1. Spain:

- 10 • Granada / Carchuna

2. Italy:

- Lazio / Sabaudia  
• Sicily / Vittoria

15 **Yield program for tomatoes:**

1. Spain:

- Granada / Salobreña  
• Cadiz / Zahora

2. Italy:

- 20 • Puglia / Palagiano

The yield program indicated splitting of trials in each crop throughout short crop cycle (spring) as well as long-term crop cycle for each country and experimental site. The selection for variety followed agronomic practice and market requests.

25

**Experimental design:**

The trial sites were selected with a history of root knot nematode population; preferably medium nematode population. To assess initial nematode population and their distribution before trial start soil samples from 4  
30 areas in the experimental field were selected. In each area, 10 vertical core subsamples, discarding the top 10 cm of soil, were sampled within the 10 to 30 cm feeder-root zone of plant, respectively.

The experimental set up was fully randomized and comprised 6 treatments with 5 replicates for each trial. Application of the formulated product PL251 (liquid formulation of *P. lilacinum* comprising BREAK-  
35 THRU® S240 and AEROSIL®) was applied sequentially with 0.75 L/ha (with  $5.4 \times 10^{10}$  viable spores /mL throughout cropping period.

Following agronomic practice one dripper per plant at two lines or one line irrigation system was set up to guarantee best chemigation and daily drip irrigation. Throughout season water amount of daily drip irrigation were adjusted according environmental conditions (air temperature °C) and developmental stage of plants (BBCH).

To keep sufficient moisture for fungal growth soil were kept moist after first application of PL 251 liquid at 14 days (d) prior to transplanting. For every further application via drip system (see Table 10A and 10B) the following chemigation cycle was used:

- 10 1/3 of the total water volume (water only)
- 1/3 of the total water volume (water + PL 251)
- 15 1/3 of the total water volume (water only)

Fertilizer management followed local recommendation and farmer practice. To guarantee best pollination for yield thus, bumble bee colonies were used in each trial and greenhouse.

20 **Table A: Treatments for cucumber trial setup:**

Treatment	Application	A	B	C	D	E	F
	Application timing	14 d prior to transplanting	at transplanting	2 d after transplanting	4 weeks after transplanting	8 weeks after transplanting	20 d after transplanting
1	UTC						
2	PL 251	0.75L/ha	0.75L/ha		0.75L/ha	0.75L/ha	
3	VELUM® SC			250 G A/ha			
4	PL 251	0.75L/ha	0.75L/ha		0.75L/ha	0.75L/ha	
4	VELUM® SC			250 G A/ha			
5	PL 251				0.75L/ha	0.75L/ha	
5	VELUM® SC			250 G A/ha			

**Table B: Treatments for tomato trial setup:**

Treatment	Application	A	B	C	D	E	F	G
	Application timing	14 d prior to transplanting	at transplanting	2 d after transplanting	12 days after transplanting	22 days after transplanting	4 weeks after transplanting	8 weeks after transplanting
1	UTC							
2	PL 251	0.75L/ha	0.75L/ha				0.75L/ha	0.75L/ha
3	VELUM <sup>®</sup> SC			250 G A/ha				
4	PL 251	0.75L/ha	0.75L/ha				0.75L/ha	0.75L/ha
4	VELUM <sup>®</sup> SC			250 G A/ha				
5	PL 251						0.75L/ha	0.75L/ha
5	VELUM <sup>®</sup> SC			250 G A/ha				

**Efficacy assessment:**

5

To investigate root galls development and efficacy of product, 15 plants were randomly selected at harvest in each plot, respectively. Hereunto, roots were digged with whole root system and washed carefully to bare root knot infestation, respectively. Based on root galls nematocidal activity was determined on the basis of the percentage (%) of gall reduction and/or damage of attack on each plant. Following assessment 100% indicate that no galls were found; 0% means that number of galls found on the roots of treated plants was equal to that in untreated control plants. Additionally crop safety was evaluated by estimating percentage of phytotoxicity on the whole plot.

10

**Yield assessment:**

For yield assessment each harvest and/or pick of fruits were recorded throughout cropping period. Pickings were executed as many times as necessary following each variety dependent characteristic. The harvest foresaw first pickings 2 weeks after last application of PL 251, respectively. At each picking date fresh weight of fruits in kilogram per plot were recorded. Further fruits were assessed by size and weight following variety characteristics.

20

## Results

### Efficacy:

PL251 solo treatment indicated reduction in gall development compared to UTC. For the cucumber trial program (FIG. 10A) efficacy for the biologic solo program showed highest efficacy in cucumber with approximately 30% compared to 24% in tomato (FIG. 10B), respectively, VELUM® SC (Fluopyram) as chemical solo program however indicated similar efficacy levels in cucumber and tomato. The sequential application of VELUM® SC and PL251 throughout cropping period clearly indicated increased efficacy values in both crops.

10

### Yield:

For yield increase significant differences in fruit weight and quantity between UTC and BIOACT® DC treated plants were observed. Statistical analysis reveals a significant improvement of fruit weight (kg) in cucumber and tomato following application of PL251, respectively. The results in both crops clearly indicate that PL 251 displays an additional plant health effect independent of its nematocidal potential leading to improved shoot weight in fruiting vegetables.

15

### Example 8: Comparison of PGP effect of different *P. lilacinum* strains

To compare the PGP effect, in particular an effect on root growth, of different *P. lilacinum* strains spore suspensions (each containing  $1 \times 10^7$  spores per ml) of three different strains were tested on tomato seedlings. Suspensions contain:

1. Sterile water
- 25 2. Spores of *Purpureocillium lilacinum* strain 251 extracted from product BioAct DC
3. Spores of *Purpureocillium lilacinum* strain extracted from product Lila-Sin WG
4. Spores of *Purpureocillium lilacinum* strain extracted from product Hocusia

Tomato seeds were placed on 1% agar plates and the plates were incubated in a vertical position in a Conviron growth chamber set for a period of 12 hrs light and 12 hrs dark, light intensity  $320 \mu\text{Mol}$ , temperature  $25^\circ\text{C}$  during the light period,  $20^\circ\text{C}$  during the dark period for 7 days. On day 7, root lengths were measured and treatments were effected afterwards (4 plantlets per treatment) by dipping the roots of the seedlings into the respective solution/suspension 1 to 4 for 15 s. The treated plantlets were placed on 1% agar plates and the roots of each plantlet were placed on the surface of the agar plates. The plates were

30

placed in the fume hood for 5 min to allow for the treatment to dry on the roots. The plates were incubated again in a vertical position in the Conviron growth chamber for another 7 days.

5 The tomato roots were analysed using the program WinRhizo which provides for a complete plant root measurement and analysis, such as length, area, volume, topology, and architecture of plant roots. Each tomato root was scanned to determine a total root surface area (in cm<sup>2</sup>) and root length (cm). The results are displayed in Figures 11A and 11B.

10 The total root length of each *Purpureocillium lilacinum* strain was evaluated. As to be seen in Figure 11A, an increase in root length (P value 0.0007) was observed with a spore suspension based on spores of *P. lilacinum* strain 251 as compared to a suspension based on *P. lilacinum* strains isolated from the products Lila-Sin and Hocusia.

15 The total root surface area of each *Purpureocillium lilacinum* strain was evaluated. As shown in Figure 11B, a significant increase in lateral root growth was only observed after treatment with *P. lilacinum* strain 251 (P value 0.0410).

### Claims

1. A method for promoting or improving plant health and/or plant growth of agricultural plants  
5 wherein the plants, the plant propagules, the seed of the plants and/or the locus where the plants are growing or are intended to grow are treated with an effective amount of a composition comprising the fungus *Purpureocillium lilacinum* or spores thereof.
2. The method of claim 1, wherein promoting or improving plant health comprises and/or manifests in  
10 improved stress tolerance, less dead basal leaves, greener leaf color, pigment content, photosynthetic activity and enhanced plant vigor.
3. The method of claim 1, wherein promoting or improving plant growth comprises or manifests in  
15 tillering increase, increase in plant height, bigger leaf blade, bigger leaf surface, stronger tillers, earlier flowering, early grain maturity, less plant verse (lodging), increased shoot growth, increased plant stand and early and better germination, earlier emergence, improved crop yield, improved total vegetative weight or whole plant biomass, improved protein content, improved oil content, improved starch content, improved root growth, improved root size, improved root weight, increased root weight, increased plant biomass and/or improved root effectiveness, improved shoot  
20 weight and improved fruit weight.
4. The method of claim 2, wherein improved stress tolerance comprises improved tolerance to drought, heat, salt, UV, water, cold and/or xenobiotic conditions.
- 25 5. The method of any one of claims 1 to 4, wherein the *P. lilacinum* strain is strain 251.
6. The method of any one of claims 1 to 5, wherein plant growth refers to root growth, root size, root weight, fruit weight, shoot weight, leaf surface, plant biomass and/or crop yield.
- 30 7. The method of any one of claims 1 to 6, wherein said promoting or improving plant health and/or plant growth is independent of pathogenic nematode pressure.
8. The method of claim 7, wherein said promoting of improving plant health and/or plant growth is in the absence of pathogenic nematode pressure.

9. The method of any one of claims 1 to 8, wherein the spores are conidia.
10. The method of any one of claims 1 to 9, wherein seed is treated.
- 5 11. The method of any one of claims 1 to 10, wherein the treatment is carried out in-furrow, by drip application, soil incorporation, drench application, sprinkler irrigation or micro injection.
12. The method of claim 11, wherein said treatment is carried out in the soil prior to germination of a seed and/or in the soil in contact with a seed or root of said plant or where a plant is intended to  
10 grow.
13. The method of any one of claims 1 to 12, wherein the treatment is carried out at least twice.
14. The method of any one of claims 1 to 12, further comprising applying, simultaneously or  
15 sequentially, at least one further plant protection agent.
15. The method of claim 13, wherein said at least one further plant protection agent is selected from the group consisting of fluopyram, *B. firmus* strain CNCM I-1582, *B. subtilis*, in particular strain QST713, abamectin, aldicarb, fenamiphos, fluensulfone, fluazaindolizine, oxamyl and a fumigant.  
20
16. The method of any one of claims 1 to 15, wherein the agricultural plant is selected from soybean, corn, wheat, triticale, barley, oat, rye, rape, millet, rice, sunflower, cotton, sugar beet, pome fruit, stone fruit, citrus, banana, strawberry, blueberry, almond, grape/grapevine, mango, papaya, peanut, potato, tomato, pepper, cucurbit, cucumber, melon, watermelon, garlic, onion, broccoli, carrot,  
25 cabbage, bean, dry bean, canola, pea, lentil, alfalfa, trefoil, clover, flax, elephant grass, grass, lettuce, sugarcane, tea, tobacco and coffee; each in its natural or genetically modified form.
17. The method of any one of claims 1 to 16, wherein the agricultural plant is tomato, cucumber or corn.  
30
18. The method of any one of claims 1 to 17, wherein said composition further comprises at least 75% polyether-modified trisiloxane.
19. The method of claim 18, wherein said polyether-modified trisiloxane is BREAK-THRU® S240.  
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20. The method of any one of claims 1 to 18, wherein said composition further comprises up to 8% fumed silica.

21. The method of claim 20, wherein said fumed silica is AEROSIL®.

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22. Use of a composition comprising the fungus *Purpureocillium lilacinum* or spores thereof for promoting or improving plant health and/or plant growth.

FIG. 1A

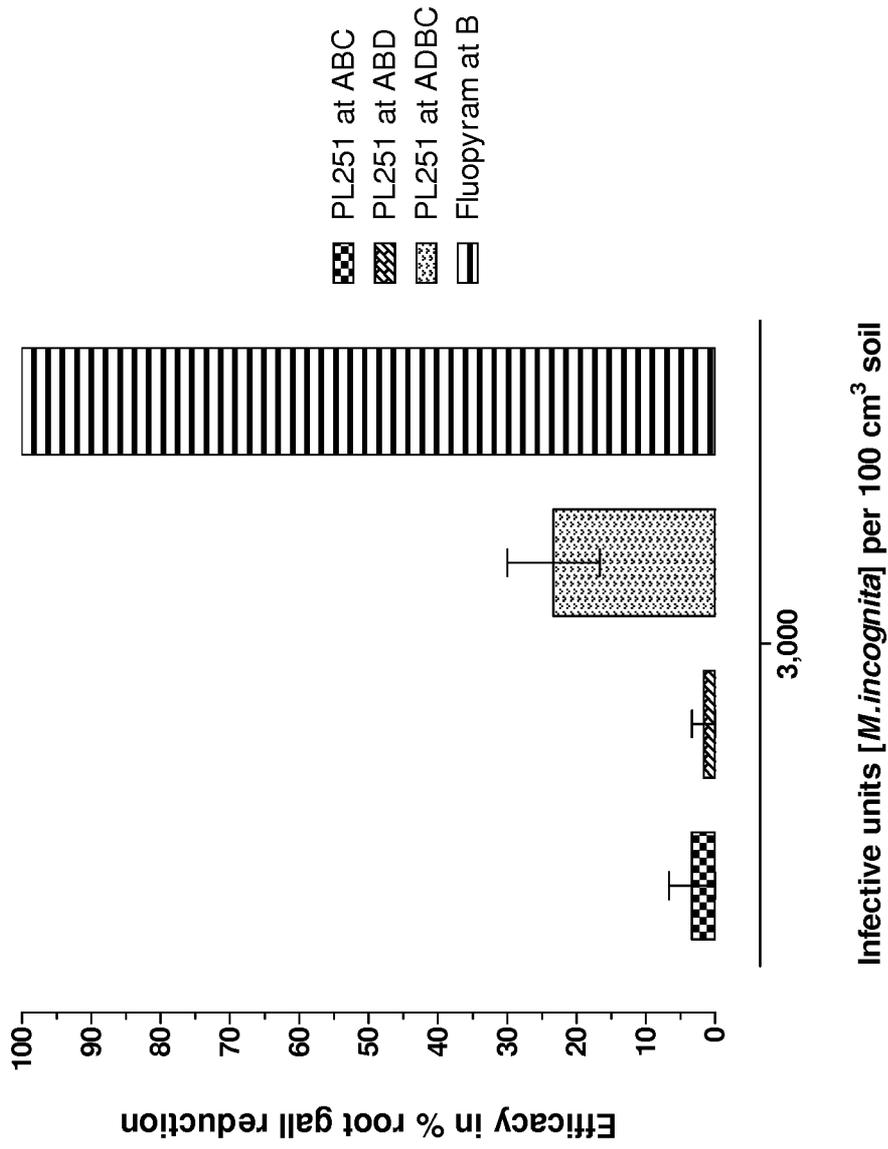


FIG. 1B

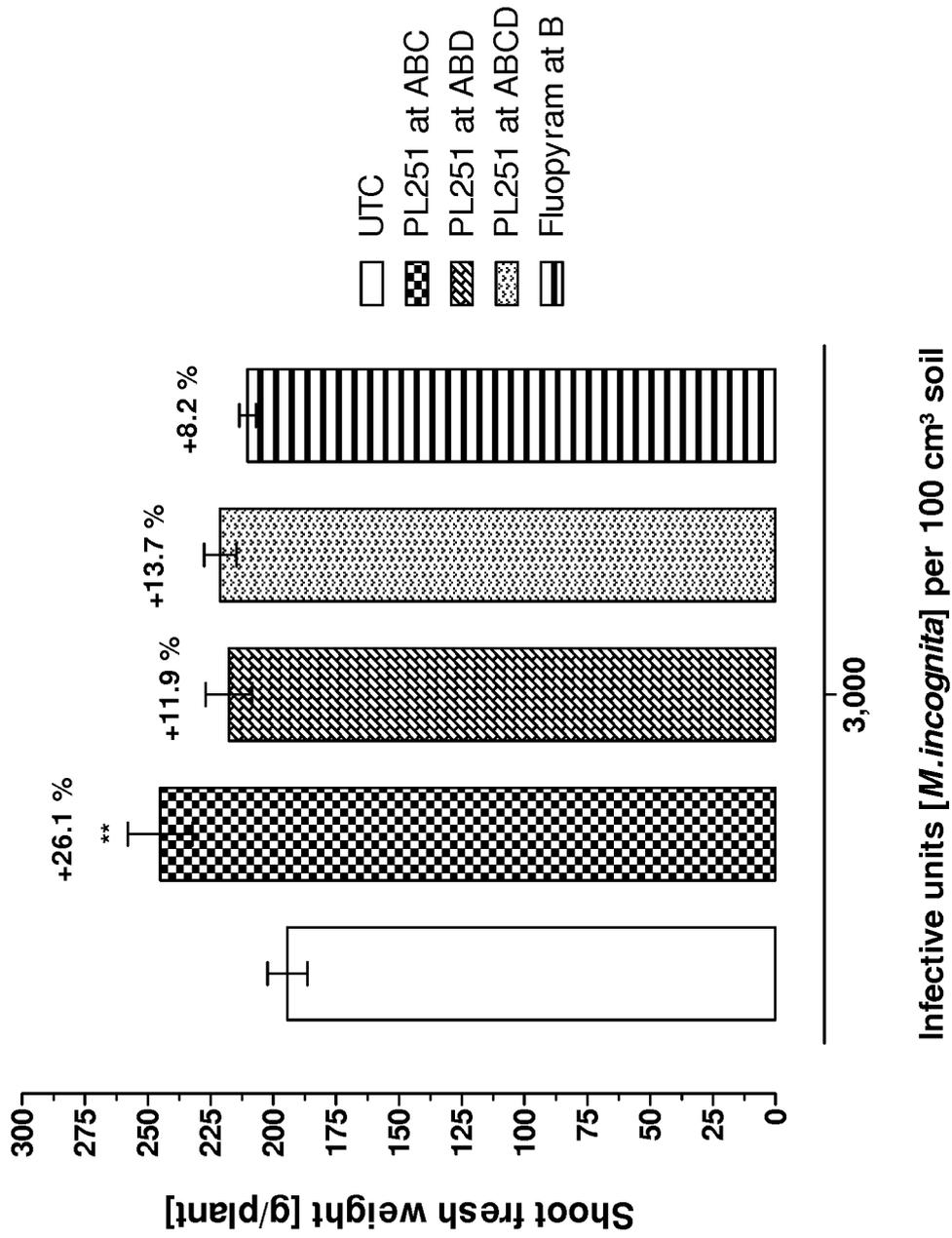


FIG. 2A

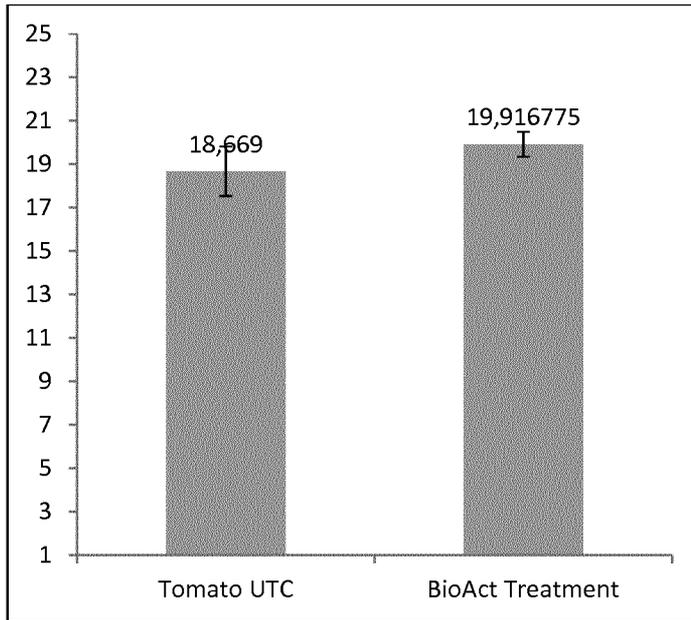


FIG. 2B

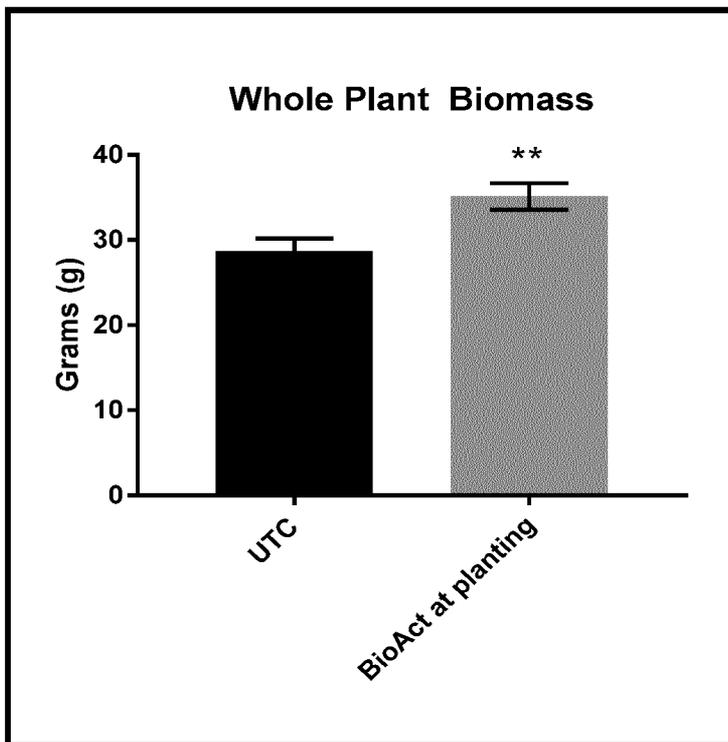


FIG. 2C

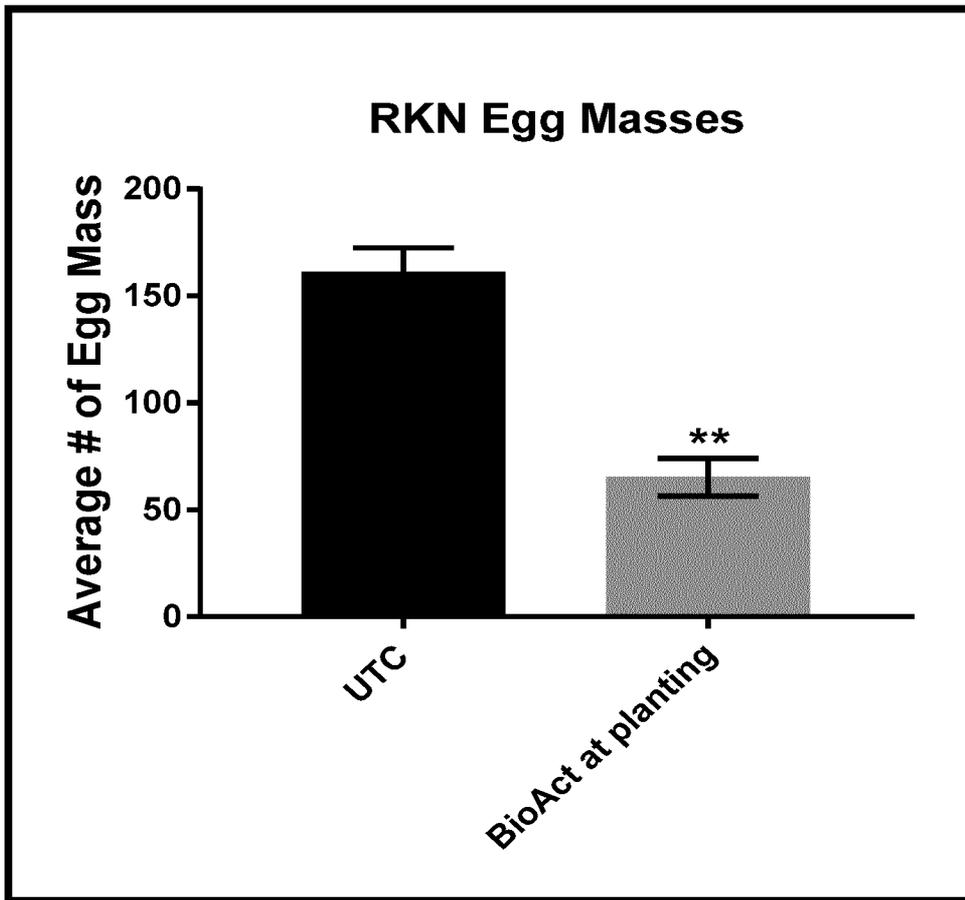
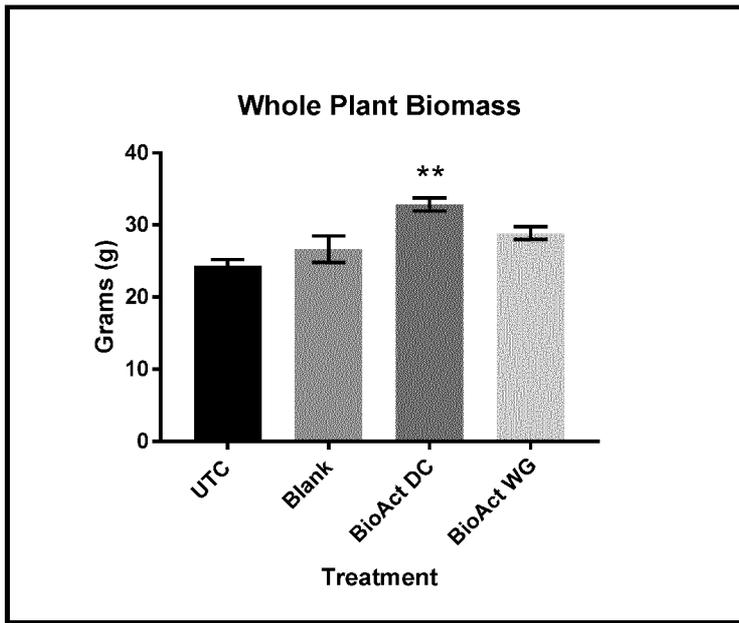


FIG. 3A



5 FIG. 3B

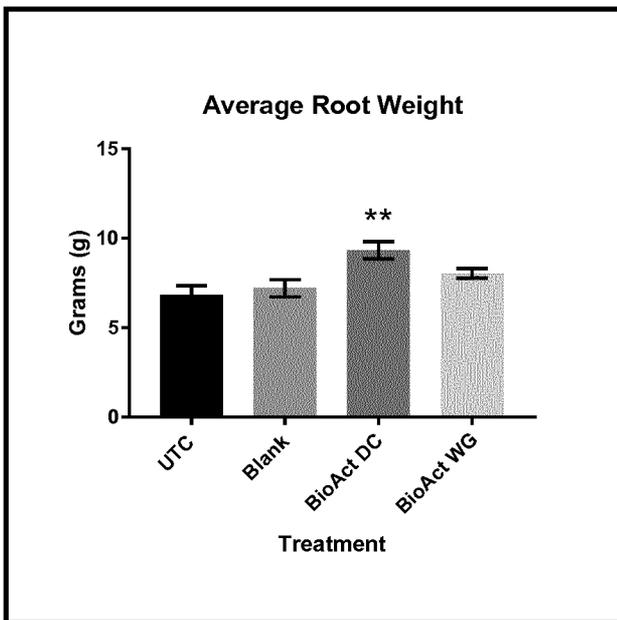


FIG. 3C

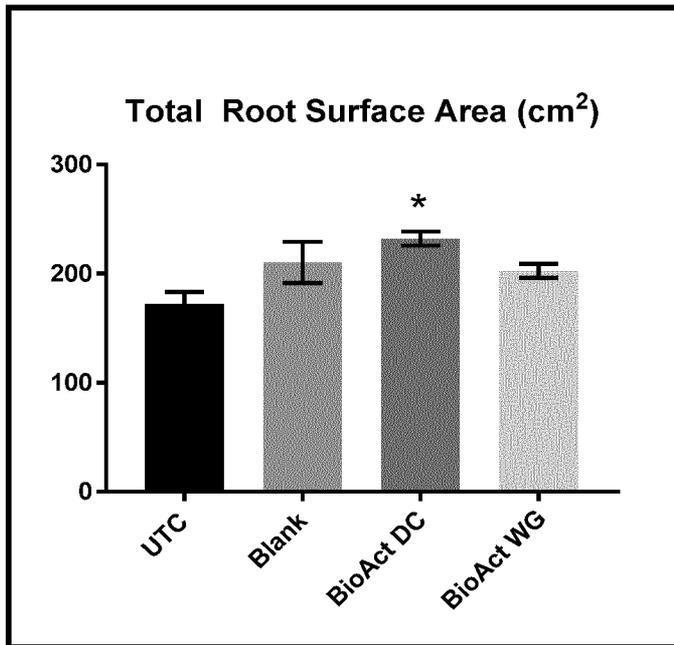


FIG. 3D

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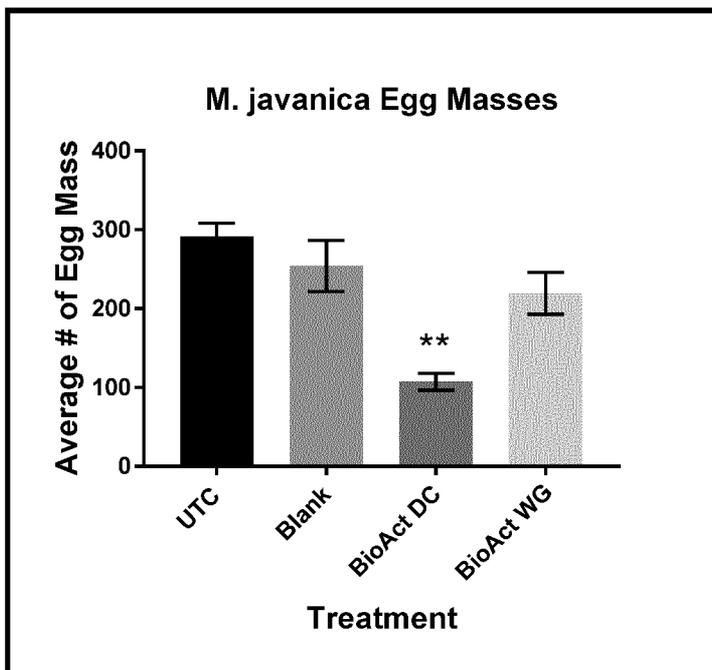


FIG. 4

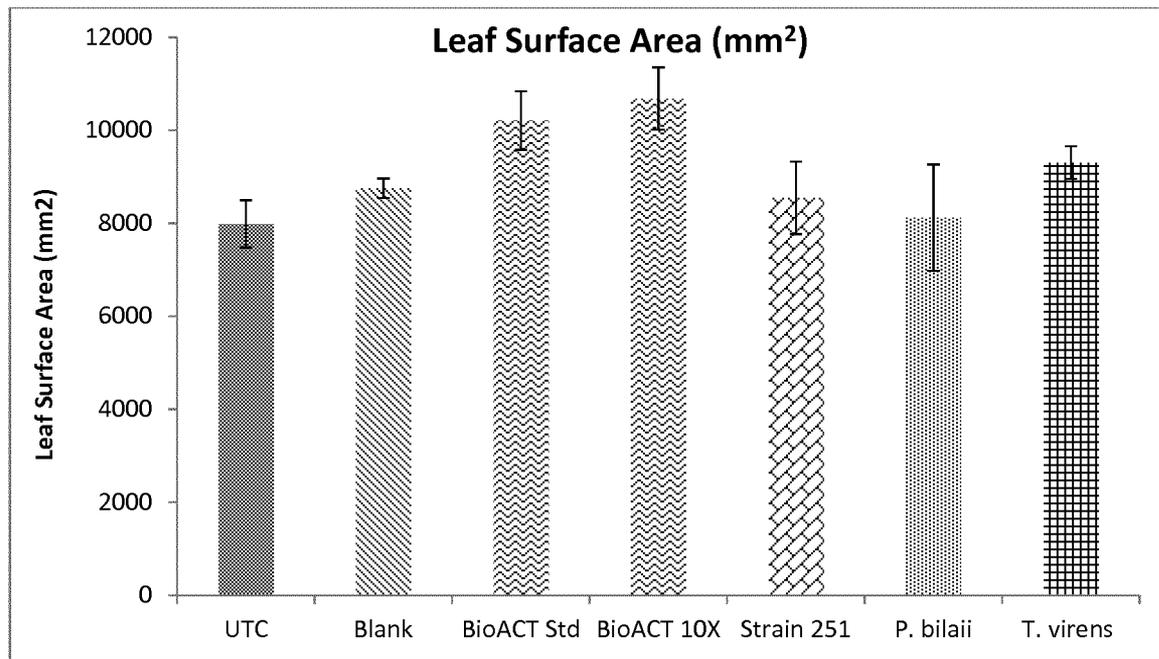


FIG. 5A

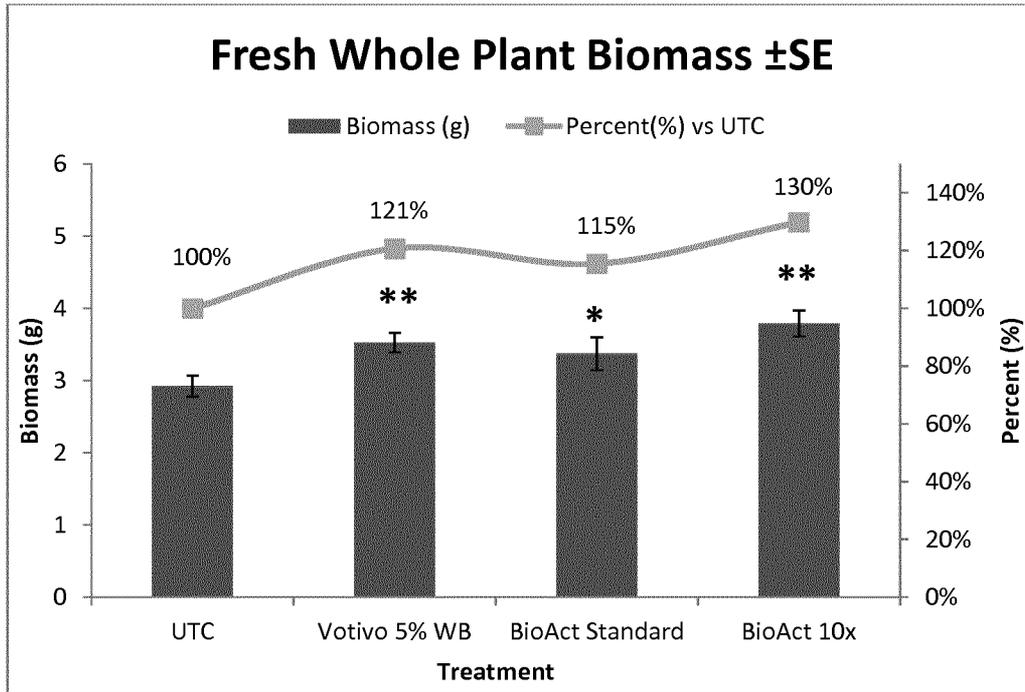
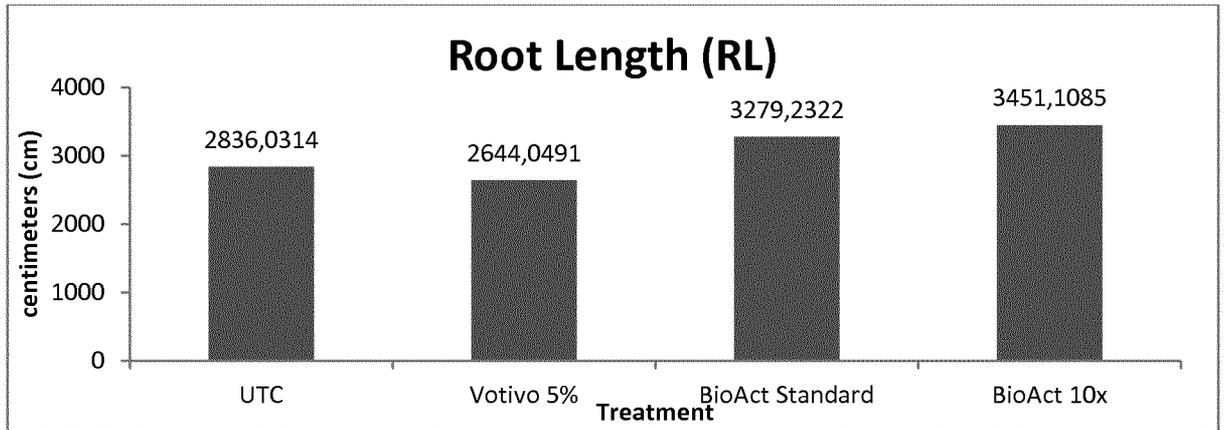


FIG. 5B



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FIG. 5C

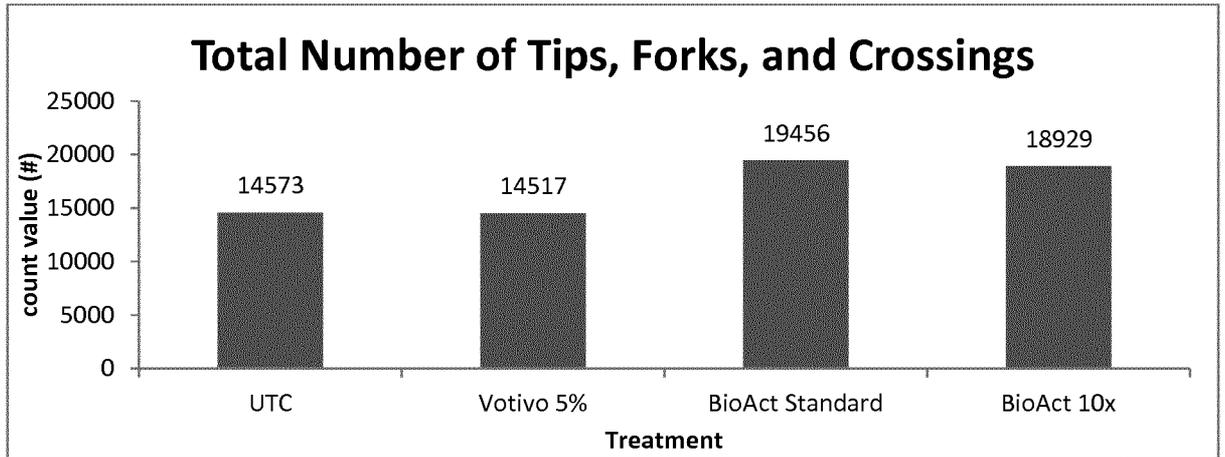
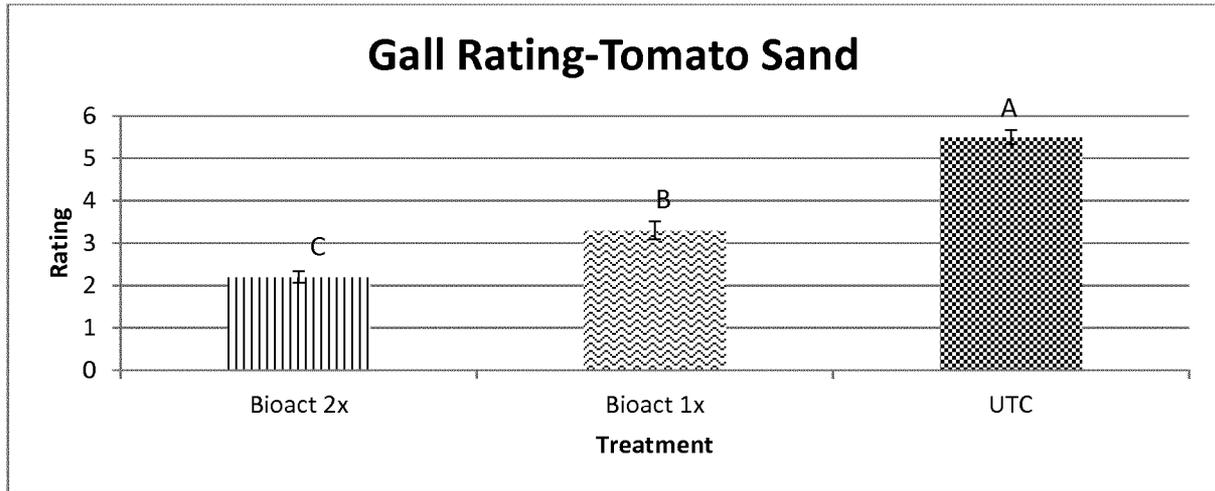


FIG. 6A



5 FIG. 6B

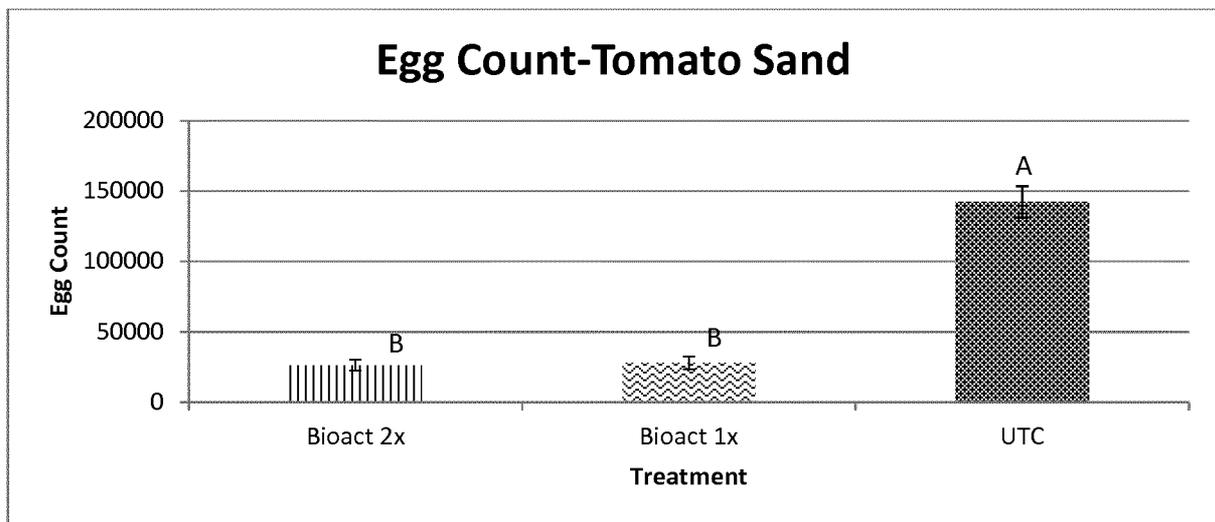
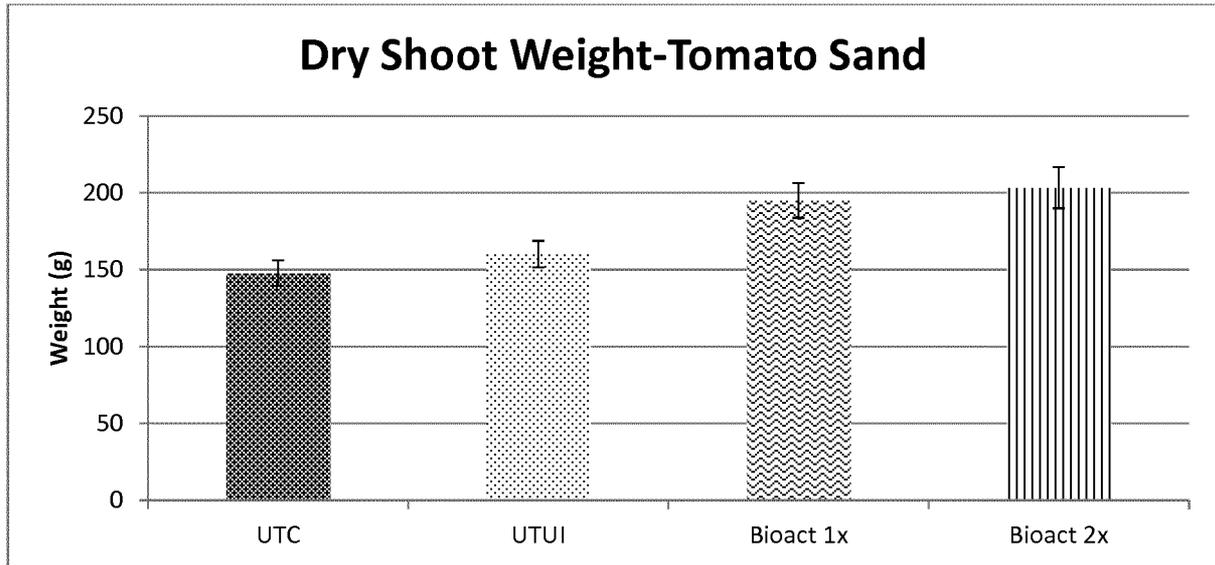


FIG. 6C



5 FIG. 6D

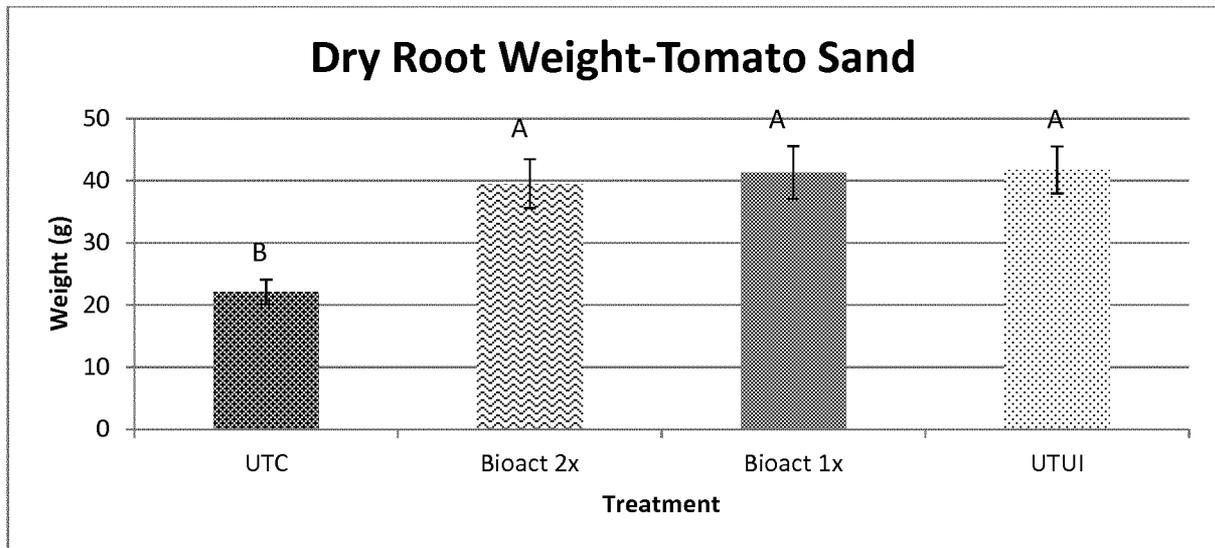


FIG. 6E

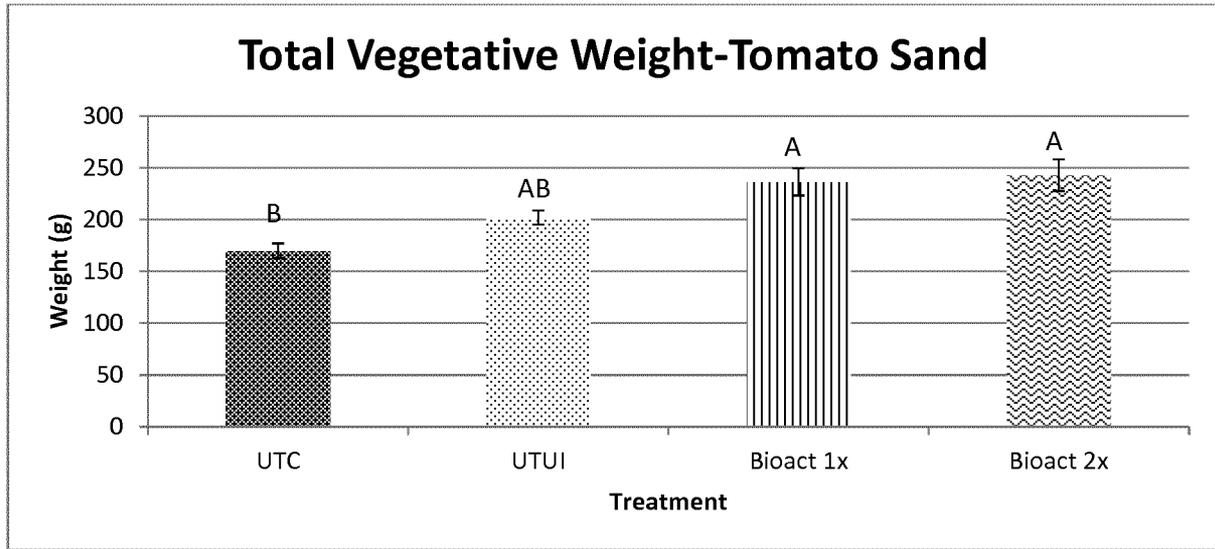
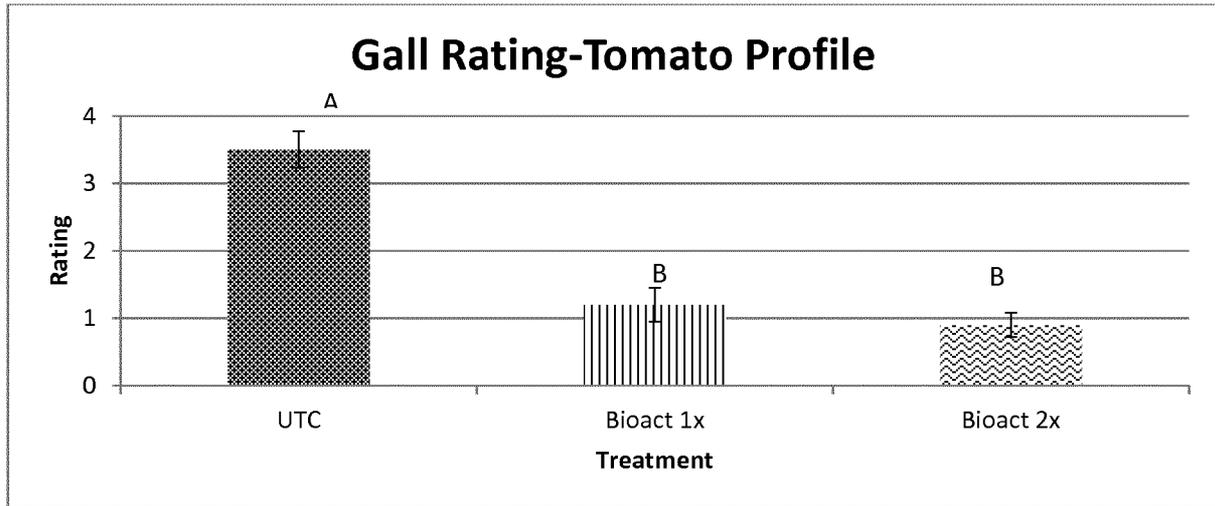
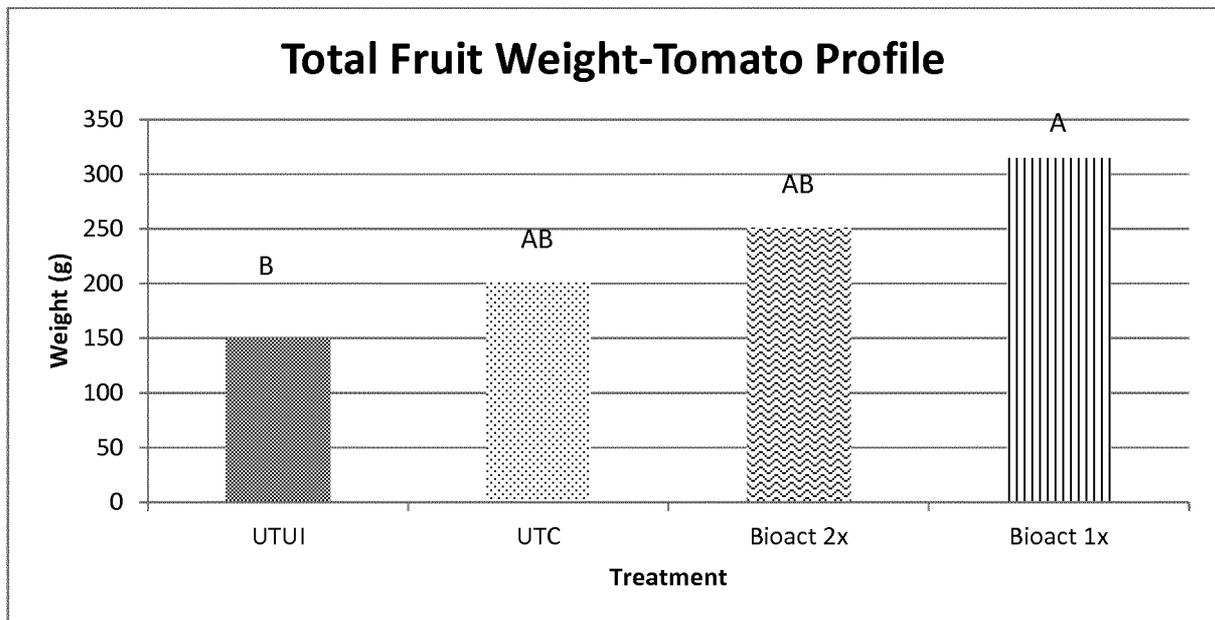


FIG. 7A



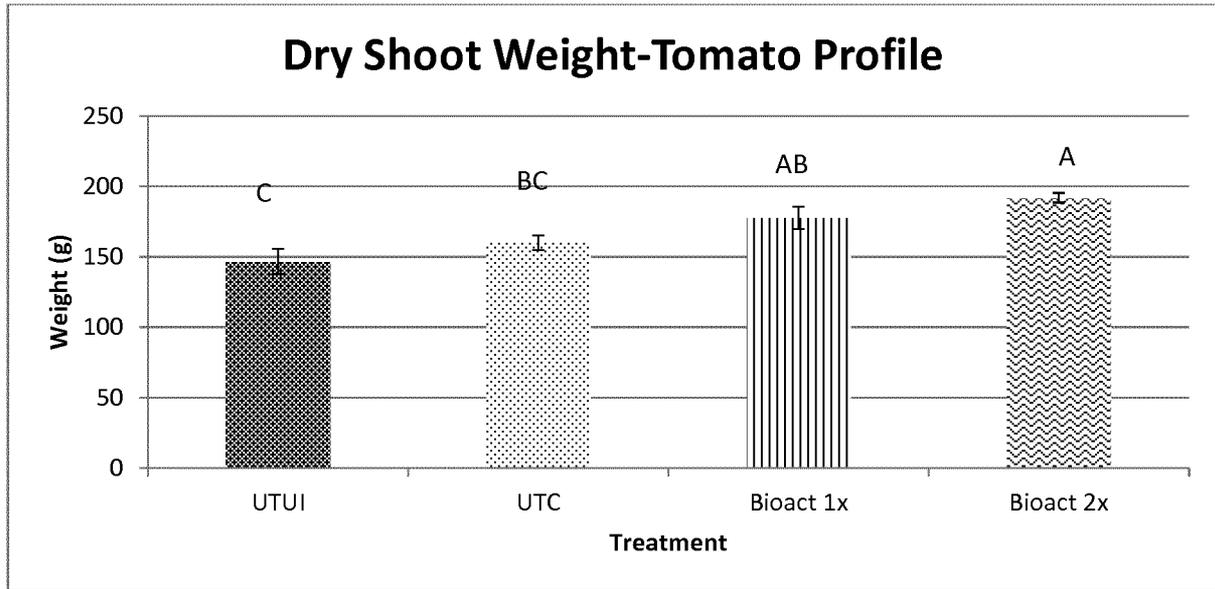
5

FIG. 7B



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FIG. 7C



5 FIG. 7D

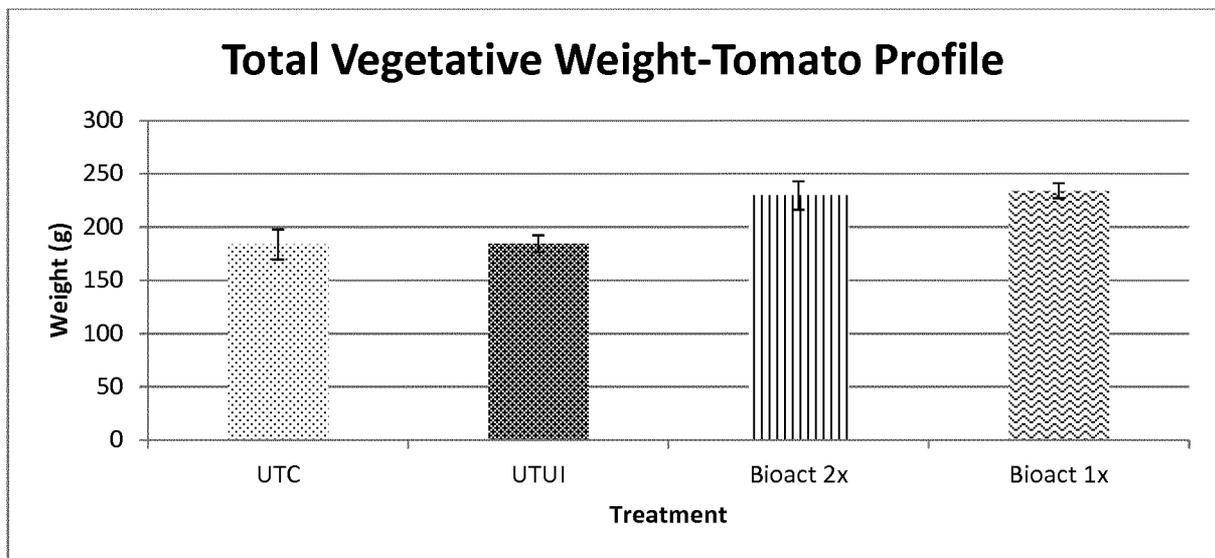
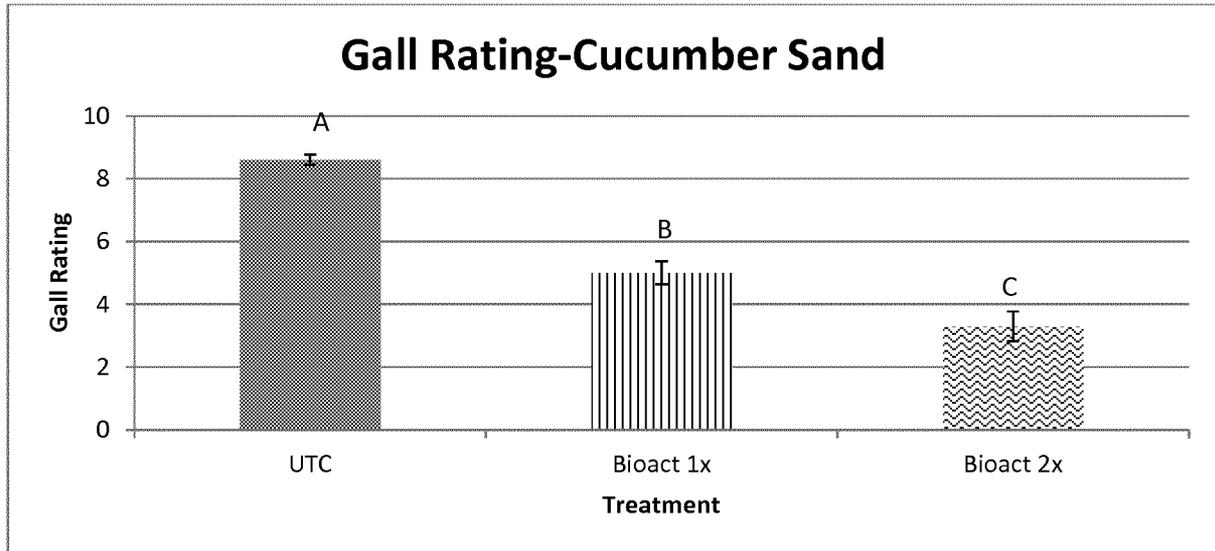


FIG. 8A



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FIG. 8B

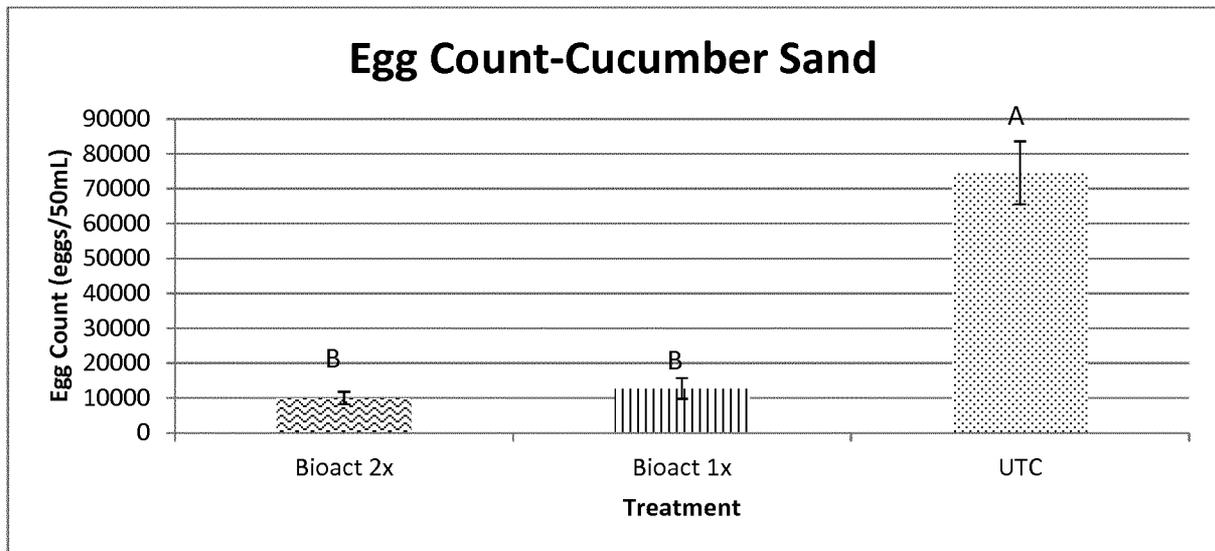
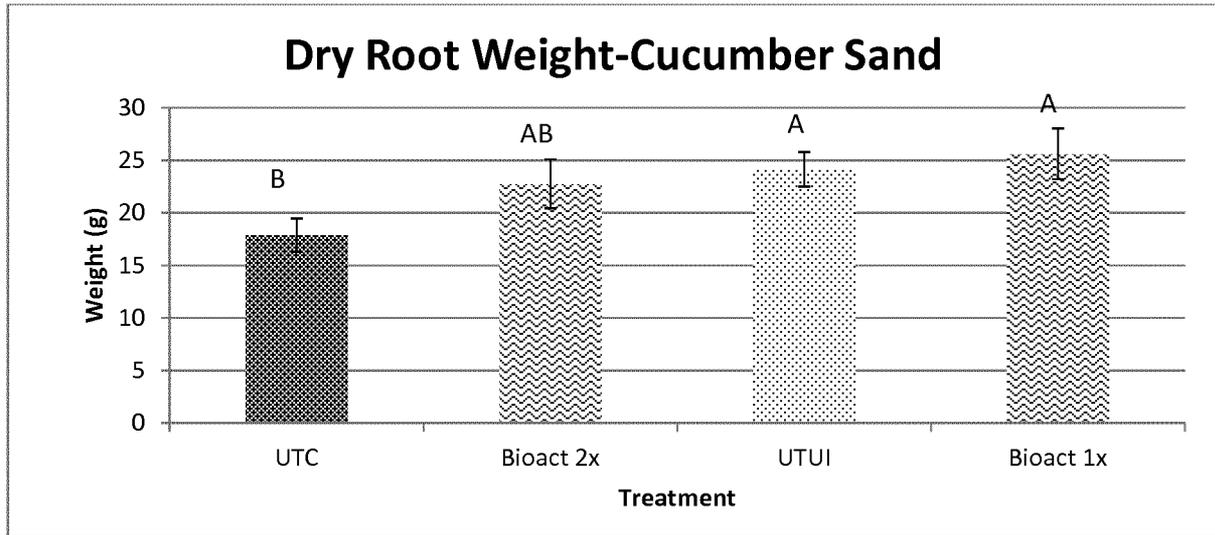


FIG. 8C



5 FIG. 9

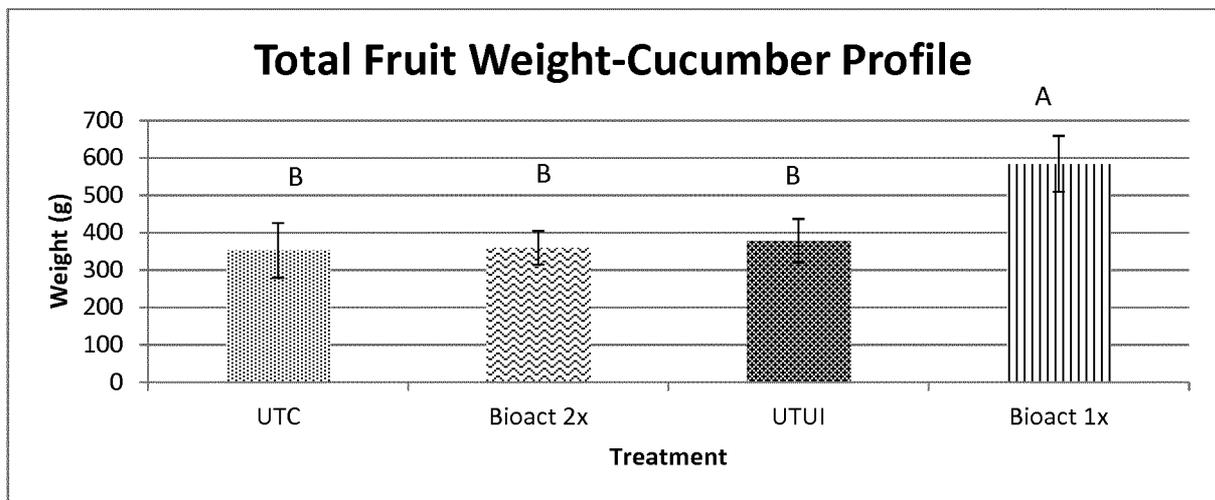
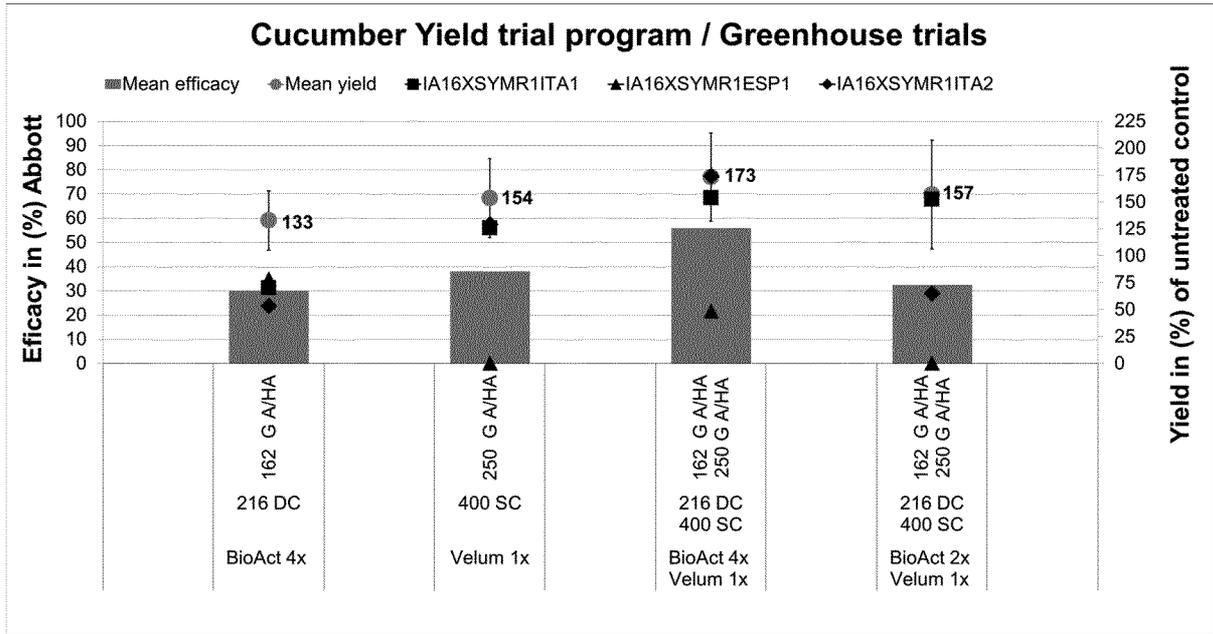
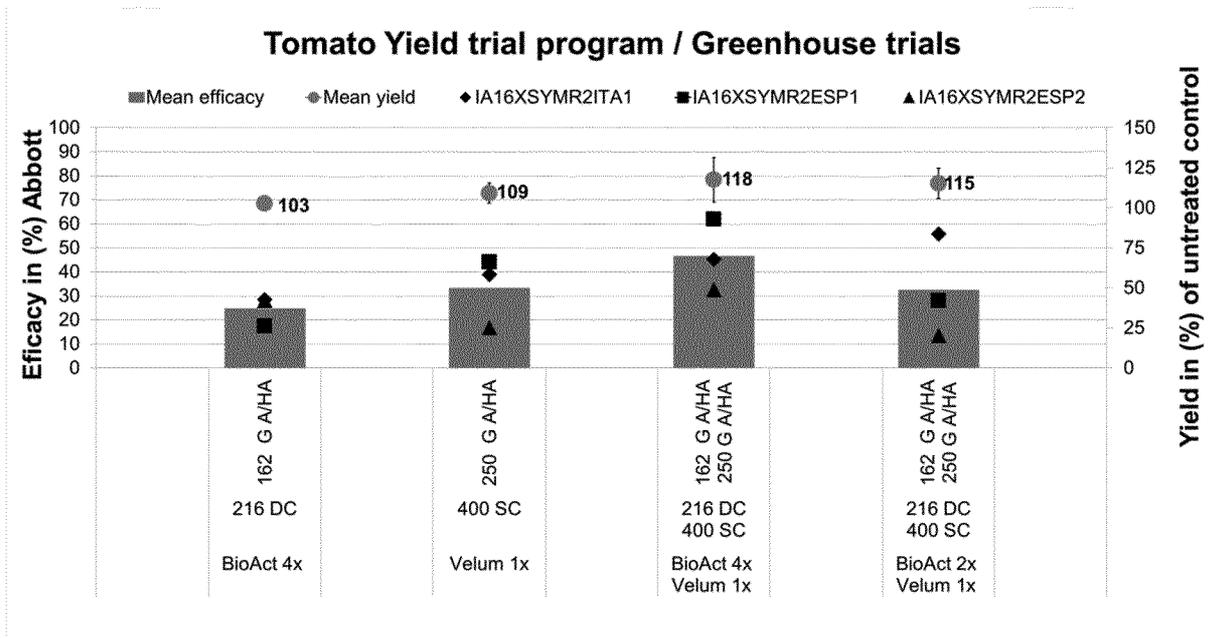


FIG. 10A



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FIG. 10B



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FIG. 11A

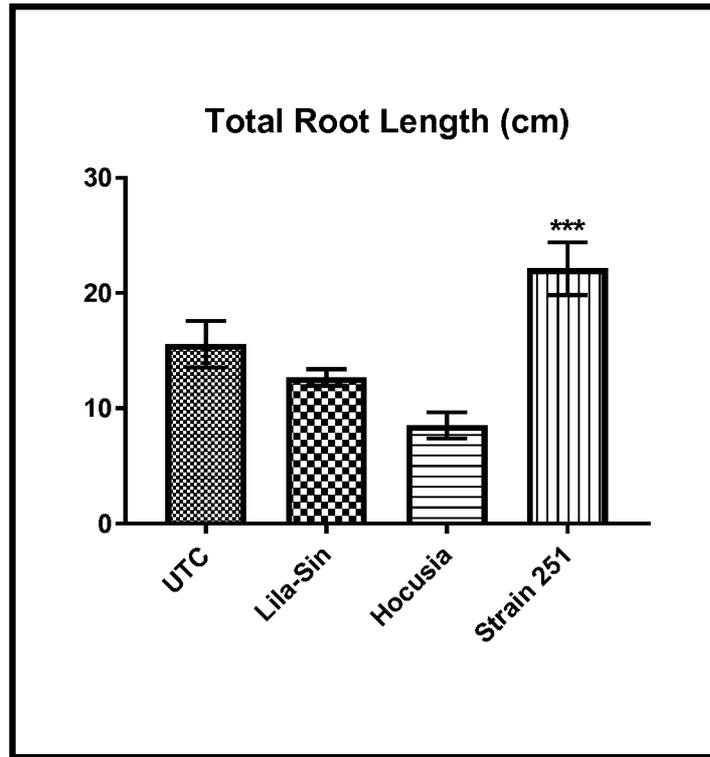
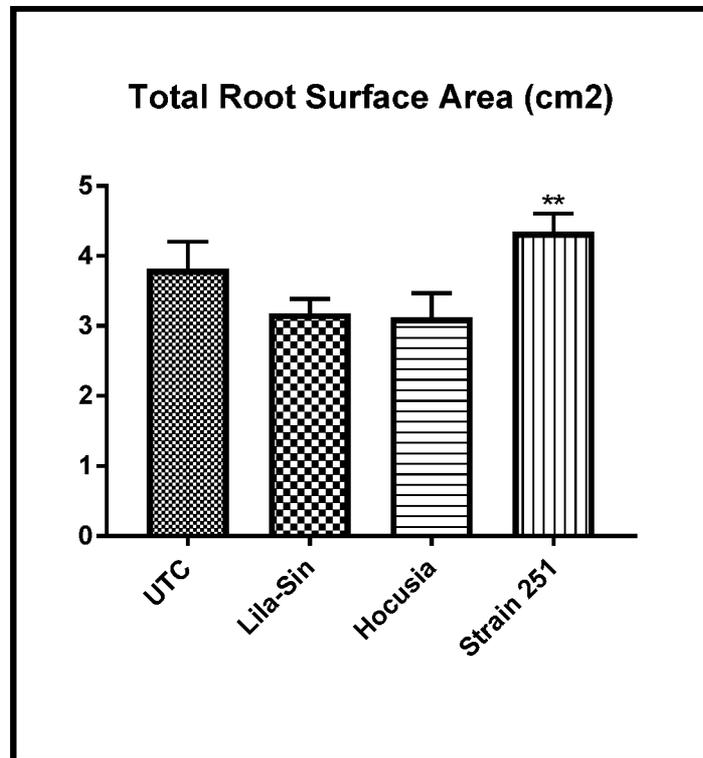


FIG. 11B



INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2017/081960

A. CLASSIFICATION OF SUBJECT MATTER  
INV. A01N63/04 A01P21/00  
ADD.  
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED  
Minimum documentation searched (classification system followed by classification symbols)  
A01N  
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	BIN GONG ET AL: "Endophytic fungus Purpureocillium sp. A5 protect mangrove plant Kandelia candel under copper stress", BRAZILIAN JOURNAL OF MICROBIOLOGY, 1 February 2017 (2017-02-01), XP055381334, BR ISSN: 1517-8382, DOI: 10.1016/j.bjm.2016.10.027 abstract figure 3 page 4, right-hand column, last paragraph	
X	CN 104 371 949 A (BEIJING LONGAGE AMMS BIOLOG TECHNOLOGY CO LTD; BEIJING LONG AGE AMMS B) 25 February 2015 (2015-02-25)	1,4,22
Y	abstract page 1, paragraph 1	1-22
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See patent family annex.

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Date of the actual completion of the international search  20 February 2018	Date of mailing of the international search report  02/03/2018
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  Galley, Carl

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2017/081960

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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Y	tables 1-1 page 18, lines 22-32	1-22
Y	----- WO 2016/193073 A1 (BAYER CROPSCIENCE AG [DE]) 8 December 2016 (2016-12-08) claims examples	10-14
Y	----- WO 2012/163322 A1 (PROPHYTA BIOLOG PFLANZENSCHUTZ GMBH [DE]; LUETH PETER [DE]) 6 December 2012 (2012-12-06) cited in the application examples	18-21
A	----- CN 104 745 483 A (SHANDONG PENGBEI BIOTECHNOLOGY CO LTD) 1 July 2015 (2015-07-01) the whole document	1-22
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Information on patent family members

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