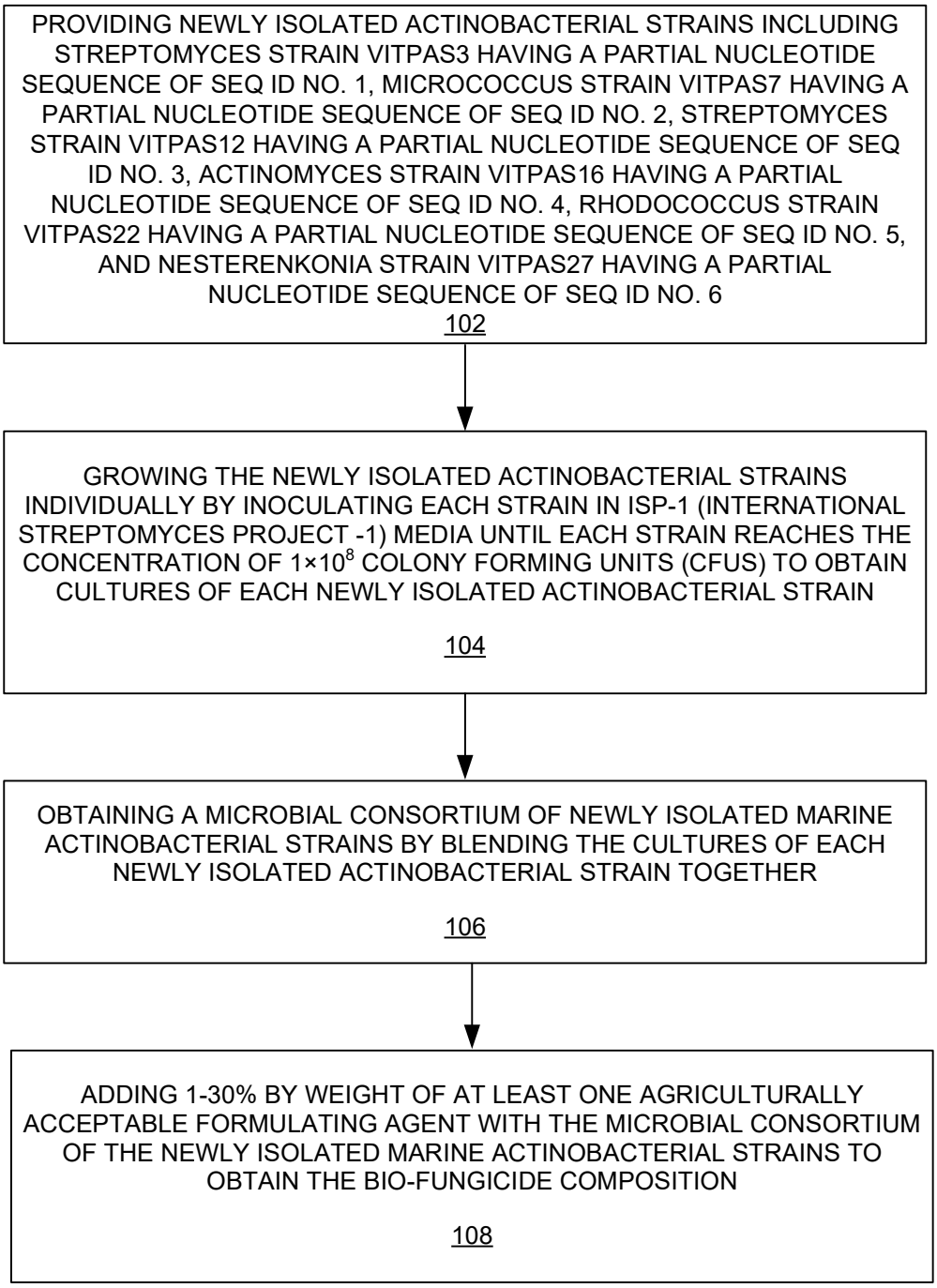

FIG. 1

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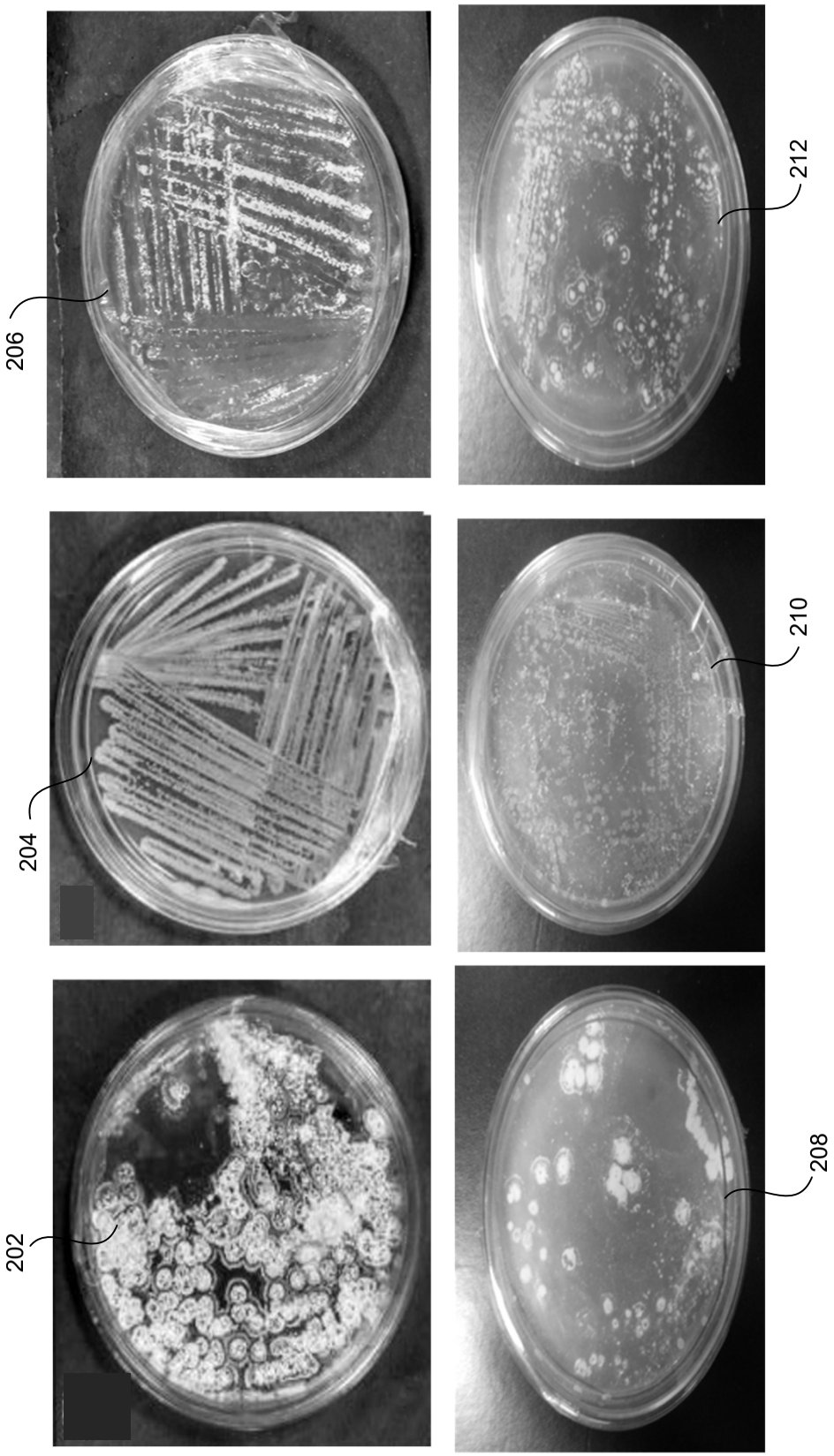

FIG. 2

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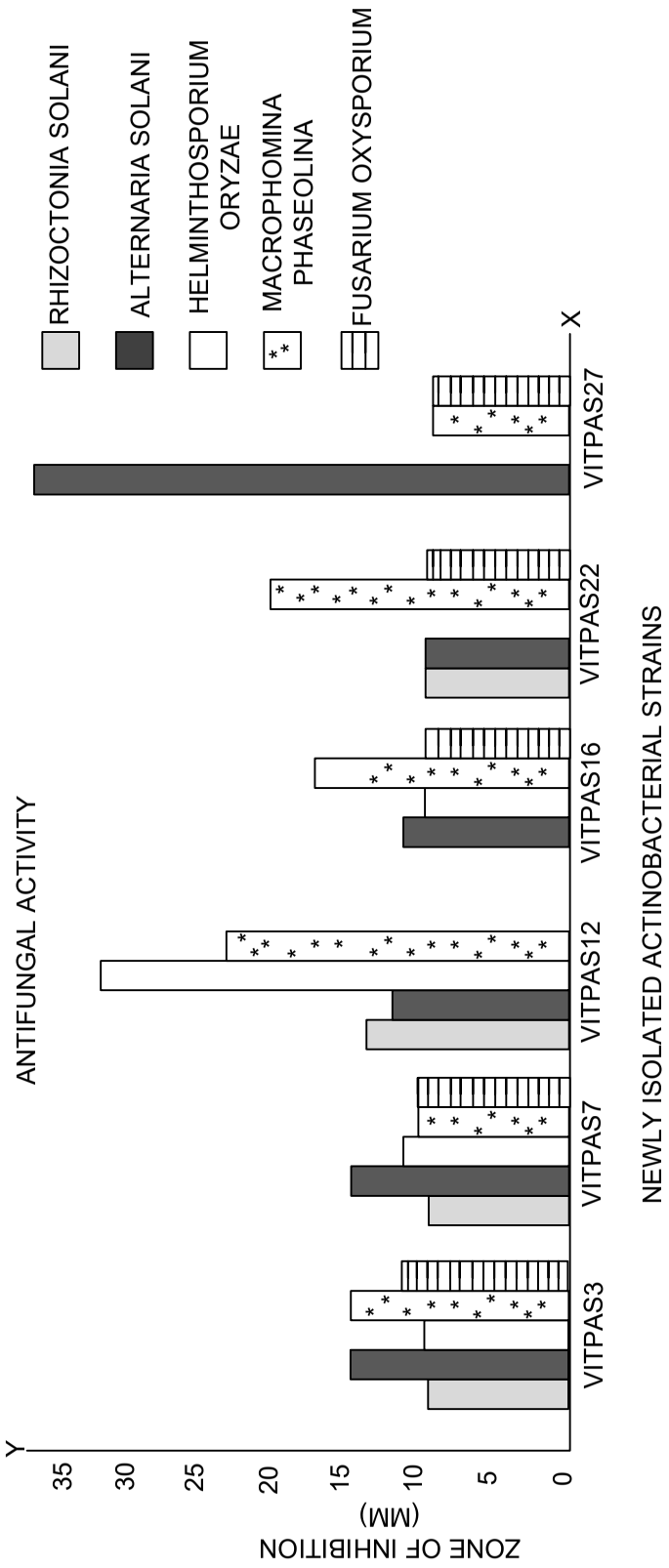

FIG. 3

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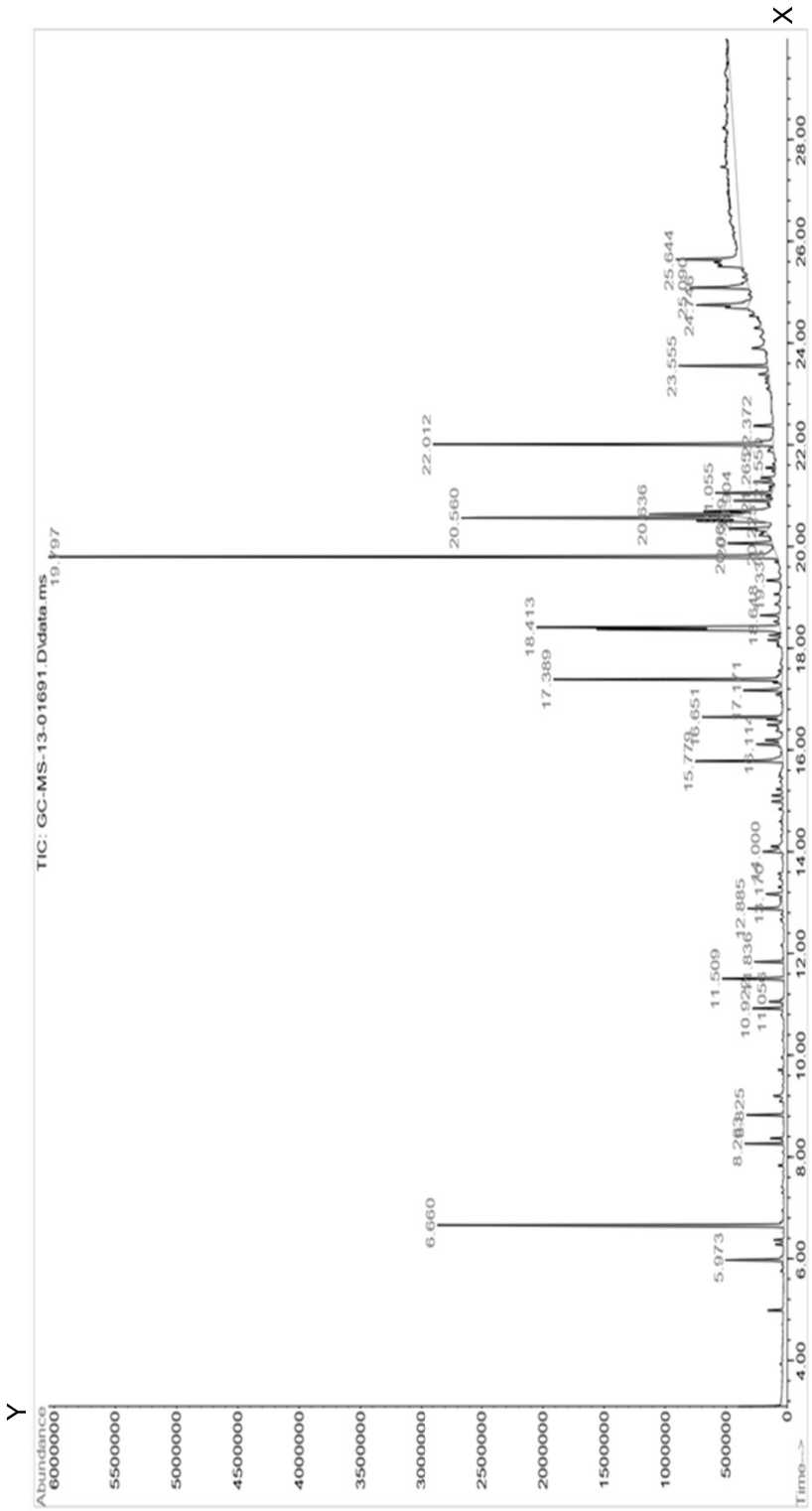

FIG. 4

SIGNATURE OF PATENT AGENT:

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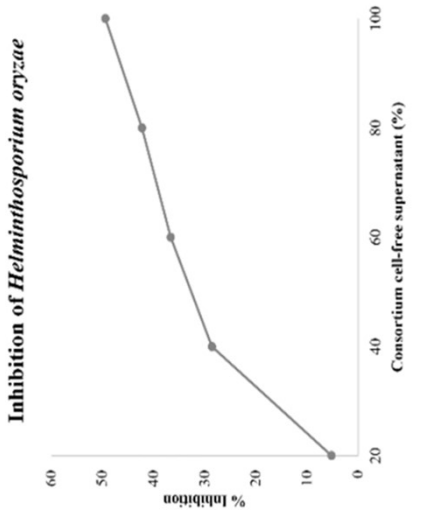

FIG. 5C

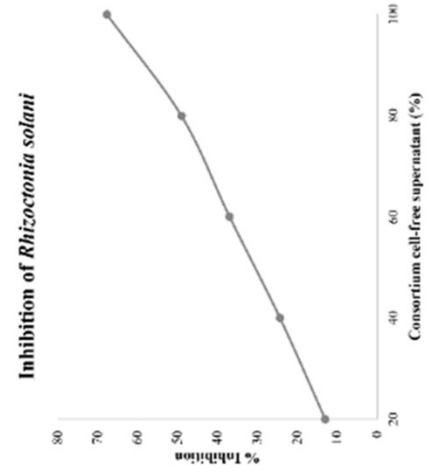

FIG. 5B

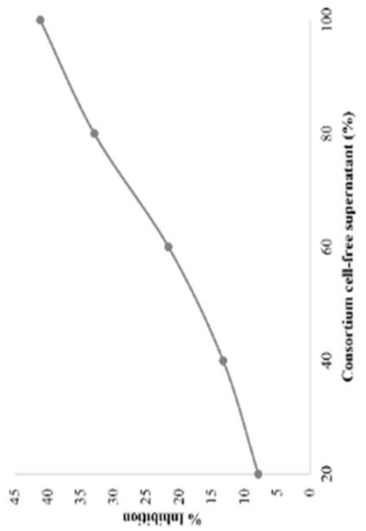

FIG. 5A

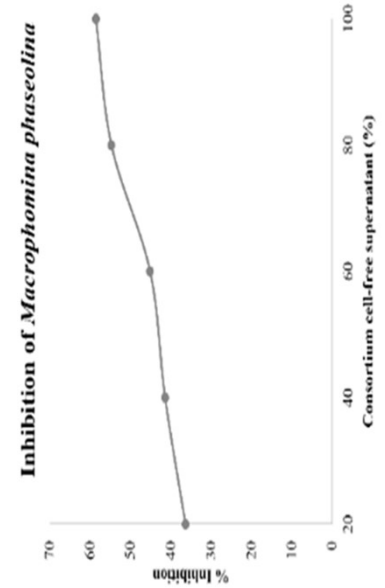

FIG. 5E

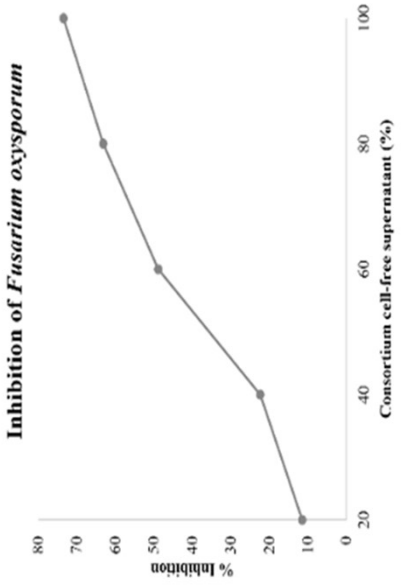

FIG. 5D

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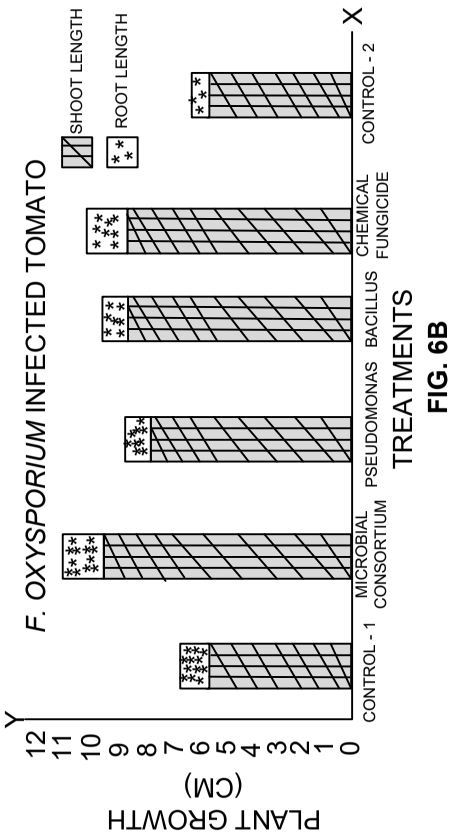

FIG. 6B

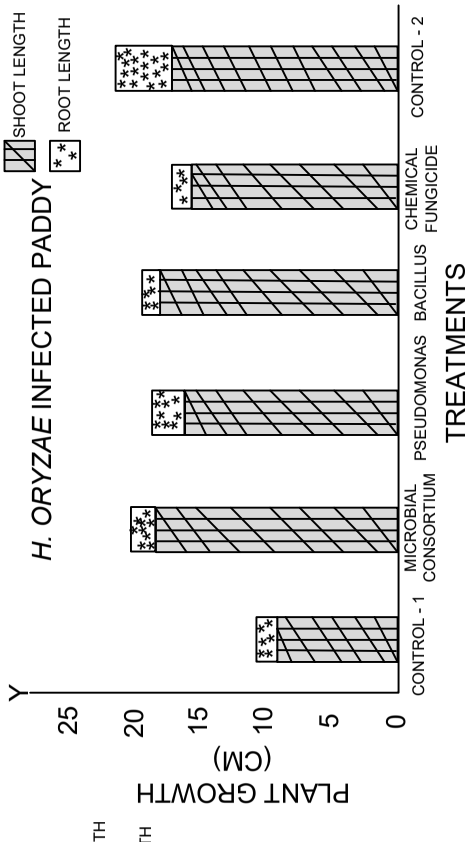

FIG. 6D

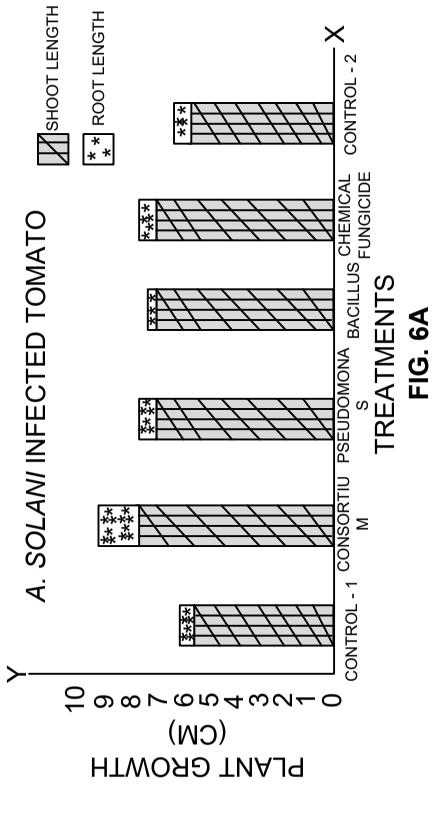

FIG. 6A

FIG. 6C

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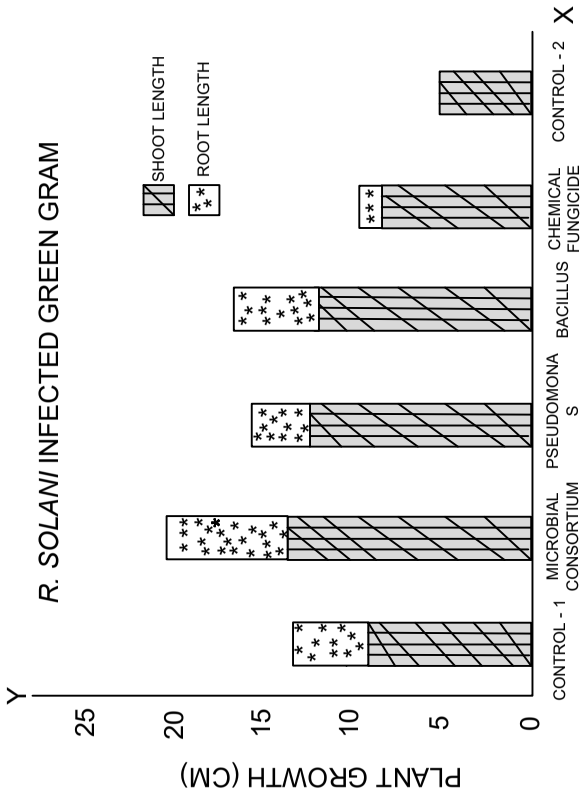

FIG. 6F

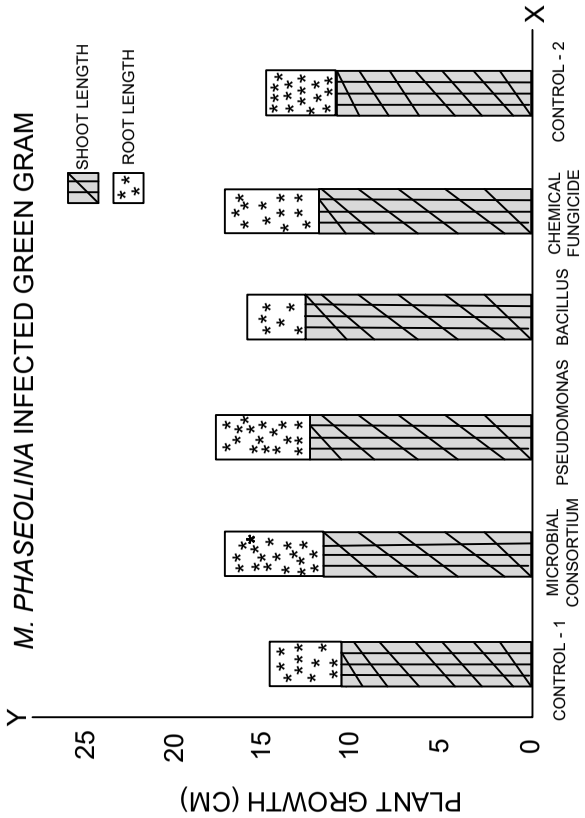

FIG. 6E

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FORM 2

THE PATENTS ACT, 1970
(39 of 1970)
&
THE PATENTS RULES, 2003
COMPLETE SPECIFICATION
(See sections 10; rule 13)

TITLE OF THE INVENTION

ACTINOBACTERIAL STRAINS-BASED BIO-FUNGICIDE COMPOSITION FOR CONTROLLING PLANT PATHOGENIC FUNGI AND METHOD FOR PRODUCING THE SAME

APPLICANT

NAME: VELLORE INSTITUTE OF TECHNOLOGY
NATIONALITY: INDIAN
ADDRESS: KATPADI, VELLORE - 632014,
TAMIL NADU, INDIA

PREAMBLE TO THE DESCRIPTION

The following specification particularly describes the invention and the manner in which it is to be performed.

ACTINOBACTERIAL STRAINS-BASED BIO-FUNGICIDE COMPOSITION FOR CONTROLLING PLANT PATHOGENIC FUNGI AND METHOD FOR PRODUCING THE SAME

BACKGROUND

Technical Field

[0001] The embodiments herein generally relate to organic pesticide compositions and more particularly, to an actinobacterial strains-based bio-fungicide composition for controlling plant pathogenic fungi. The embodiments further relate to a method of producing the bio-fungicide composition.

Description of the Related Art

[0002] India is one of the emerging nations with the fifth largest economy globally, where the Indian agriculture sector contributes ~20% of the total Indian Gross Domestic Product (GDP) (Food and Agriculture Organization (FAO), 2021). Almost ~70% of the Indian population depends on agriculture for their livelihood, with 197.6 million hectares of the total cropped area, which produced 315.7 million tons of food grain during 2021-22 (Annual Reports, 2022). In addition, India's population is ~1.4 billion, expected to rise to 1.67 billion by 2050. Plants are the only food source for the human population, and impact on plant growth due to climatic changes, growth conditions, and disease infection can cause serious effects such as food scarcity and inflation.

[0003] Among the above-mentioned factors, disease infection poses a particularly significant threat to global food security. Plant diseases, whether caused by fungi, bacteria, viruses, or other pathogens, can devastate crops, leading to reduced yields, lower quality produce, and even crop failure. Among disease infection, fungal pathogens are particularly significant contributors to reduced plant growth and crop yield losses. The impact of fungal diseases on plants is exacerbated by changing climatic conditions, which can create favorable environments for these pathogens to thrive. As a result, addressing fungal disease infections in plants is of paramount importance for ensuring a stable and abundant food supply, as unchecked infections can lead to food scarcity, inflated prices, and overall food security concerns.

[0004] The current scenario of plant disease management highly depends on using chemical pesticides and fungicides, which cause adverse effects on the plants, environment, livestock, and humans. Further, these fungicides are expensive and are not readily available to farmers. Moreover, these fungicides exhibit poor effectiveness, limited pathogen coverage, and often require multiple applications, resulting in high costs and potential fungicide resistance. Hence, the development of organic bio-fungicides in agriculture is of paramount importance.

[0005] Research on the development of organic bio-fungicides has been notably limited, hindering progress and leaving a significant knowledge gap. While some conventional organic bio-fungicides are available in the market, they still face challenges related to efficacy, pathogen coverage limitations, and higher production cost.

[0006] Accordingly, there remains a need to address the aforementioned technical drawbacks in existing techniques in developing fungicides at low cost with improved efficacy and broad pathogen coverage.

SUMMARY

[0007] In view of a foregoing, an embodiment herein provides a bio-fungicide composition for controlling plant pathogenic fungi and promoting plant growth. The bio-fungicide composition includes a microbial consortium of two or more newly isolated marine actinobacterial strains that includes (i) Streptomyces strain VITPAS3 that includes a gene sequence encoding 16S ribosomal RNA (rRNA) having a partial nucleotide sequence of SEQ ID NO: 1; (ii) Micrococcus strain VITPAS7 that includes a gene sequence encoding 16S rRNA having a partial nucleotide sequence of SEQ ID NO: 2; (iii) Streptomyces strain VITPAS12 that includes a gene sequence encoding 16S rRNA having a partial nucleotide sequence of SEQ ID NO: 3; (iv) Actinomyces strain VITPAS16 that includes a gene sequence encoding 16S rRNA having a partial nucleotide sequence of SEQ ID NO: 4; (v) Rhodococcus strain VITPAS22 that includes a gene sequence encoding 16S rRNA having a partial nucleotide sequence of SEQ ID NO: 5; and (vi) Nesterenkonia strain VITPAS27 that includes a gene sequence encoding 16S rRNA having a partial nucleotide sequence of SEQ ID NO: 6. The newly isolated marine actinobacterial strains are combined together to form the bio-fungicide composition that provides a synergetic anti-fungal activity to control the plant pathogenic fungi and plant growth-promoting properties when contacting with at least one of seeds, plants or plant parts.

[0008] In some embodiments, a concentration of each marine actinobacterial strain in the microbial consortium is 1×10^8 colony forming units (CFUs). A viable stock concentration of the microbial consortium is 6×10^8 CFUs.

[0009] In some embodiments, the bio-fungicide composition further includes at least one agriculturally acceptable formulating agent that includes a carrier an adjuvant, and an excipient. A concentration of at least one agriculturally acceptable formulating agent is in a range between 1-30% by weight.

[0010] In some embodiments, the microbial consortium is formulated into at least one form of liquid, liquid concentrates, solid granules, powder, emulsions, paste, semi-solid formations, wettable powders, or seed coatings using the at least one pharmaceutically acceptable formulating agent.

[0011] In some embodiments, the bio-fungicide composition is formulated into a foliar spray composition that includes 6×10^8 CFUs of the microbial consortium including 1×10^8 CFUs of Streptomyces strain VITPAS3; 1×10^8 CFUs of Micrococcus strain VITPAS7; 1×10^8 CFUs of Streptomyces strain; 1×10^8 CFUs of Actinomyces strain VITPAS16; 1×10^8 CFUs of Rhodococcus strain VITPAS22; and 1×10^8 CFUs of Nesterenkonia strain VITPAS27, and 10% volume by volume (V/v) of glycerol. In some embodiments, 6 mL of the foliar spray composition is diluted with 1000 mL of water.

[0012] In some embodiments, the bio-fungicide composition is formulated into a root fungicide composition that includes 6×10^8 CFUs of the microbial consortium including 1×10^8 CFUs of Streptomyces strain VITPAS3; 1×10^8 CFUs of Micrococcus strain VITPAS7; 1×10^8 CFUs of Streptomyces strain; 1×10^8 CFUs of Actinomyces strain VITPAS16; 1×10^8 CFUs of Rhodococcus strain VITPAS22; and 1×10^8 CFUs of Nesterenkonia strain VITPAS27, and wood charcoal carrier. A ratio between the microbial consortium and the wood charcoal carrier is of 1:2.

[0013] In some embodiments, the bio-fungicide composition is formulated into a seed coating composition that includes 6×10^8 CFUs of the microbial consortium including 1×10^8 CFUs of Streptomyces strain VITPAS3; 1×10^8 CFUs of Micrococcus strain VITPAS7; 1×10^8 CFUs of Streptomyces strain; 1×10^8 CFUs of Actinomyces strain VITPAS16; 1×10^8 CFUs of Rhodococcus strain VITPAS22; and 1×10^8 CFUs of Nesterenkonia strain VITPAS27, and 3% weight by volume (W/v) of sodium alginate cross linked with calcium chloride ($CaCl_2$).

[0014] In some embodiments, the bio-fungicide composition controls the plant pathogenic fungi that includes *Alternaria solani*, *Rhizoctonia solani*, *Macrophomina phaseolina solani*, *Cochliobolus miyabeanus*, and *Fusarium oxysporum.*

[0015] In one aspect, a method of producing a bio-fungicide composition for controlling plant pathogenic fungi and promoting plant growth is provided. (i) providing newly isolated actinobacterial strains including (a) Streptomyces strain VITPAS3 that includes a gene sequence encoding 16S ribosomal RNA (rRNA) having a partial nucleotide sequence of SEQ ID NO: 1; (b) Micrococcus strain VITPAS7 that includes a gene sequence encoding 16S rRNA having a partial nucleotide sequence of SEQ ID NO: 2; (c) Streptomyces strain VITPAS12 that includes a gene sequence encoding 16S rRNA having a partial nucleotide sequence of SEQ ID NO: 3; (d) Actinomyces strain VITPAS16 that includes a gene sequence encoding 16S rRNA having a partial nucleotide sequence of SEQ ID NO: 4; (e) Rhodococcus strain VITPAS22 that includes a gene sequence encoding 16S rRNA having a partial nucleotide sequence of SEQ ID NO: 5; and (f) Nesterenkonia strain VITPAS27 that includes a gene sequence encoding 16S rRNA having a partial nucleotide sequence of SEQ ID NO: 6; (ii) obtaining cultures of each newly isolated actinobacterial strain by inoculating each strain in International Streptomyces project -1 (ISP-1) media to grow until each strain reaches a concentration of 1×10^8 colony forming units (CFUs); (iii) obtaining a microbial consortium of the newly isolated marine actinobacterial strains by evenly blending the cultures of each newly isolated actinobacterial strain together. A viable stock concentration of the microbial consortium is 6×10^8 CFUs; and (iv) adding 1-30% by weight of at least one agriculturally acceptable formulating agent with the microbial consortium of the newly isolated marine actinobacterial strains to obtain the bio-fungicide composition. The bio-fungicide composition provides a synergetic anti-fungal activity to control the plant pathogenic fungi and plant growth-promoting properties when contacting with at least one of seeds, plants or plant parts.

[0016] In some embodiments, the microbial consortium of the newly isolated marine actinobacterial strains is stable at a temperature in a range between 10°C and 45°C, a pH in a range between 4 and 9, and a salt concentration in a range of from 1% to 21% NaCl. In some embodiments, the microbial consortium is storable at 27°C for 9 months and 4°C for 18 months.

[0017] The newly isolated actinobacterial strains in the bio-fungicide composition produce one or more substances such as antifungal agents, antibacterial agents, and plant growth-promoting substances, which can prevent the plants from pathogenic infections caused by fungal pathogens and promote the growth of plants. Thus, the bio-fungicide composition of the present disclosure provides the anti-fungal activity and plant growth-promoting properties. Further, the bio-fungicide composition has a broader spectrum of activity, showing anti-fungal activity towards a wide range of plant pathogens. Hence, the bio-fungicide composition can be applied to various crops targeting various plant pathogens, thereby increasing efficacy in disease management than conventional fungicides that target only one pathogen and plant.

[0018] As the bio-fungicide composition of the present disclosure is produced from the microbial consortium of actinobacterial strains, the bio-fungicide composition is organic in nature. Hence, the bio-fungicide composition is environmentally friendly and does not have adverse effects on the environment, including soil, water, and non-target organisms. Further, the bio-fungicide composition is safe for humans and livestock, thereby reducing health risks for farmers and consumers. Moreover, the bio-fungicide composition is designed to be cost-effective, making it more accessible to farmers. As the microbial consortium of the bio-fungicide composition can tolerate high salt, acidic/alkaline pH, and temperature, the bio-fungicide composition of the present disclosure can be applied in harsh environmental conditions, such as fields with hard water, high salinity, and acidic and alkaline pH. The bio-fungicide composition requires lesser dosage and fewer applications compared to the conventional pesticides.

[0019] These and other aspects of the embodiments herein will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following descriptions, while indicating preferred embodiments and numerous specific details thereof, are given by way of illustration and not of limitation. Many changes and modifications may be made within the scope of the embodiments herein without departing from the spirit thereof, and the embodiments herein include all such modifications.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The embodiments herein will be better understood from the following detailed description with reference to the drawings, in which:

[0021] FIG. 1 is a flow diagram that illustrates a method of producing a bio-fungicide composition for controlling plant pathogenic fungi and promoting plant growth, according to some embodiments herein;

[0022] FIG. 2 is an exemplary view that illustrates newly isolated actinobacterial strains that are isolated from marine sediment samples, according to some embodiments herein;

[0023] FIG. 3 is a graphical representation that illustrates antifungal activity of each newly isolated actinobacterial strain on plant pathogenic fungi, according to some embodiments herein;

[0024] FIG. 4 is a graphical representation that illustrates a chromatogram of a microbial consortium that is analyzed using Gas Chromatography and Mass Spectrometry according to some embodiments herein;

[0025] FIGS. 5A-5E are graphical representations that illustrate antifungal activity and Minimum Inhibitory Concentration (MIC) of a microbial consortium of the newly isolated actinobacterial strains on plant pathogenic fungi, according to some embodiments herein;

[0026] FIG. 6A is a graphical representation that illustrates shoot length and root length of *Alternaria solani*-infected tomato plants that are treated with different fungicides according to some embodiments herein.;

[0027] FIG. 6B is a graphical representation that illustrates shoot length and root length of *Fusarium oxysporum*-infected tomato plants that are treated with different fungicides according to some embodiments herein;

[0028] FIG. 6C is a graphical representation that illustrates shoot length and root length of *Rhizoctonia solani*-infected paddy plants that are treated with different fungicides according to some embodiments herein;

[0029] FIG. 6D is a graphical representation that illustrates shoot length and root length of *Helminthosporium oryzae*-infected paddy plants that are treated with different fungicides according to some embodiments herein;

[0030] FIG. 6E is a graphical representation that illustrates shoot length and root length of *Macrophomina phaseolina*-infected green gram plants that are treated with different fungicides according to some embodiments herein; and

[0031] FIG. 6F is a graphical representation that illustrates shoot length and root length of green gram plants that are grown from seed infected with *Rhizoctonia solani* according to some embodiments herein.

DETAILED DESCRIPTION OF THE DRAWINGS

[0032] The embodiments herein and the various features and advantageous details thereof are explained more fully with reference to the non-limiting embodiments that are illustrated in the accompanying drawings and detailed in the following description. Descriptions of well-known components and processing techniques are omitted so as to not unnecessarily obscure the embodiments herein. The examples used herein are intended merely to facilitate an understanding of ways in which the embodiments herein may be practiced and to further enable those of skill in the art to practice the embodiments herein. Accordingly, the examples should not be construed as limiting the scope of the embodiments

herein.

[0033] As mentioned, there remains a need to develop fungicides at low cost with improved efficacy and broad pathogen coverage. Various embodiments disclosed herein provide a bio-fungicide composition that includes one or more newly isolated actinobacterial strains for controlling plant pathogenic fungi. The bio-fungicide composition of the present disclosure provides a synergetic anti-fungal activity to control the plant pathogenic fungi. Further, the bio-fungicide composition of the present disclosure has plant growth-promoting properties to improve plant growth and yield when contacting with plants or plant parts. That is, the newly isolated actinobacterial strains produce one or more substances such as antifungal agents, antibacterial agents, and plant growth-promoting substances, which can prevent the plants from pathogenic infections caused by fungal pathogens and promote the growth of plants. Thus, the bio-fungicide composition of the present disclosure provides the anti-fungal activity and plant growth-promoting properties.

[0034] Referring now to the drawings, and more particularly to FIGS. 1 through 6F, where similar reference characters denote corresponding features consistently throughout the figures, preferred embodiments are shown.

[0035] Embodiments herein disclose a bio-fungicide composition for controlling plant pathogenic fungi and promoting plant growth. The bio-fungicide composition includes a microbial consortium of two or more newly isolated marine actinobacterial strains. The newly isolated marine actinobacterial strains includes (i) Streptomyces strain VITPAS3; (ii) Micrococcus strain VITPAS7; (iii) Streptomyces strain VITPAS12; (iv) Actinomyces strain VITPAS16; (v) Rhodococcus strain VITPAS22; and (vi) Nesterenkonia strain VITPAS27.

[0036] All the newly isolated marine actinobacterial strains (VITPAS3, VITPAS7, VITPAS12, VITPAS16, VITPAS22, and VITPAS27) are isolated from the marine sediment samples collected from the Vhan island of Tamil Nadu, India. The newly isolated actinobacterial strains are confirmed using 16S rRNA gene sequence analysis and gene sequence information of the actinobacterial strains is deposited in the National Centre for Biotechnology Information (NCBI) database.

[0037] The actinobacterial strain VITPAS3 is identified as Streptomyces species and includes a gene sequence encoding 16S ribosomal RNA (rRNA) that includes a partial nucleotide sequence of SEQ ID NO: 1. The nucleotide sequence of SEQ ID NO: 1 is deposited in NCBI with an accession number: OK036589.1. The actinobacterial strain VITPAS7 is identified as Micrococcus species and includes a gene sequence encoding 16S rRNA that includes a partial nucleotide sequence of SEQ ID NO: 2. The nucleotide sequence of SEQ ID NO: 2 is deposited in NCBI with an accession number: MH041154.1. The actinobacterial strain VITPAS12 is identified as Streptomyces species and includes a gene sequence encoding 16S rRNA that includes a partial nucleotide sequence of SEQ ID NO: 3. The nucleotide sequence of SEQ ID NO: 3 is deposited in NCBI with an accession number: OK036602.1. The actinobacterial strain VITPAS16 is identified as Streptomyces species and includes a gene sequence encoding 16S rRNA that includes a partial nucleotide sequence of

SEQ ID NO: 4. The nucleotide sequence of SEQ ID NO: 4 is deposited in NCBI with an accession number: OK036597.1. The actinobacterial strain VITPAS22 is identified as Streptomyces species and includes a gene sequence encoding 16S rRNA that includes a partial nucleotide sequence of SEQ ID NO: 5. The nucleotide sequence of SEQ ID NO: 5 deposited in NCBI with an accession number: OK036625.1. The actinobacterial strain VITPAS27 is identified as Streptomyces species and includes a gene sequence encoding 16S rRNA that includes a partial nucleotide sequence of SEQ ID NO: 6. The nucleotide sequence of SEQ ID NO: 6 is deposited in NCBI with an accession number: OK036630.1. All the newly isolated actinobacterial strains are maintained and used in pure or substantially pure form to form the bio-fungicide composition. The newly isolated actinobacterial strains may be used as viable spores or colonies.

[0038] In some embodiments, the bio-fungicide composition includes the microbial consortium of (i) Streptomyces strain VITPAS3 having the partial nucleotide sequence of SEQ ID NO: 1; (ii) Micrococcus strain VITPAS7 having the partial nucleotide sequence of SEQ ID NO: 2; (iii) Streptomyces strain VITPAS12 having the partial nucleotide sequence of SEQ ID NO: 3; (iv) Actinomyces strain VITPAS16 having the partial nucleotide sequence of SEQ ID NO: 4; (v) Rhodococcus strain VITPAS22 having the nucleotide sequence of SEQ ID NO: 5; and (vi) Nesterenkonia strain VITPAS27 having the partial nucleotide sequence of SEQ ID NO: 6. A concentration of each actinobacterial strain in the microbial consortium is $1\text{-}2\times10^{5\text{-}8}$ colony forming units (CFUs). In some embodiments, each actinobacterial strain is in the concentration of 1×10^{8} CFUs in the microbial consortium. In some embodiments, a viable stock concentration of the microbial consortium is 6×10^{8} CFUs.

[0039] The microbial consortium of the newly isolated marine actinobacterial strains is stable at a temperature in a range between 10 °C and 45°C, a pH in a range between 4 and 9, and a salt concentration in a range of from 1% to 21% sodium chloride (NaCl), and storable at 27°C for 9 months and 4°C for 18 months.

[0040] The bio-fungicide composition further includes one or more agriculturally acceptable formulating agents. The agriculturally acceptable formulating agent may include a carrier, an adjuvant or a dilutant, and an excipient. The carrier may be selected from a group consisting of biochar, wood charcoal, ash, activated charcoal, perlite, vermiculate, farmyard manure, food waste manure, vermicompost, neem cake, and a combination thereof. The dilutant or adjuvant may be selected from a group consisting of glycerol, polysorbate 20, polysorbate 80, polyvinylpyrrolidone, carboxymethylcellulose, trehalose, sugar fatty acid esters, esterified vegetable oils, clove oil, chilli oil, ginger oil, lemongrass oil, rosemary oil, neem oil, or a combination thereof. The excipient may be selected from a group consisting of agar, sodium alginate, extra cellular polysaccharides (EPS), xanthan gum, gum arabic, gellan gum, and a combination thereof. A concentration of the one or more agriculturally acceptable formulating agents is in a range between 1-30% by weight.

[0041] In some embodiments, the microbial consortium is formulated into at least one form of liquid, liquid concentrates, solid granules, powder, emulsions, paste, semi-solid

formations, wettable powders, or seed coatings using the one or more pharmaceutically acceptable formulating agents, resulting in formation of the bio-fungicide composition. The bio-fungicide composition may be used for foliar application, root or soil application, or seed coating application.

[0042] The bio-fungicide composition may be applied to seeds, plants or plant parts. In some embodiments, the bio-fungicide composition may be sprayed directly on the plant parts including the leaves, flowers, stems, branches, pods, roots and fruits. In some embodiments, the bio-fungicide composition may be applied directly to the soil around the root zone of the plants. In some embodiments, the bio-fungicide composition may be applied with seed as seed coatings.

[0043] In some embodiments, the bio-fungicide composition is formulated into a foliar spray composition that includes 6×10^8 CFUs of the microbial consortium including 1×10^8 CFUs of Streptomyces strain VITPAS3; 1×10^8 CFUs of Micrococcus strain VITPAS7; 1×10^8 CFUs of Streptomyces strain; 1×10^8 CFUs of Actinomyces strain VITPAS16; 1×10^8 CFUs of Rhodococcus strain VITPAS22; and 1×10^8 CFUs of Nesterenkonia strain VITPAS27, and 10% glycerol. All the strains are equally mixed along with the glycerol to obtain a stock microbial consortium. That is, the stock microbial consortium may contain 6×10^8 CFUs in 10% volume by volume (V/v) of `glycerol. In some embodiments, 6 mL of the stock microbial consortium (6×10^8 CFUs) is diluted with 1000 mL of water to achieve a final microbial consortium concentration of 1×10^8 CFUs and applied as foliar treatment to the seeds, plant or plant parts.

[0044] In some embodiments, the bio-fungicide composition is formulated into a root fungicide composition that includes 6×10^8 CFUs of the microbial consortium including 1×10^8 CFUs of Streptomyces strain VITPAS3; 1×10^8 CFUs of Micrococcus strain VITPAS7; 1×10^8 CFUs of Streptomyces strain; 1×10^8 CFUs of Actinomyces strain VITPAS16; 1×10^8 CFUs of Rhodococcus strain VITPAS22; and 1×10^8 CFUs of Nesterenkonia strain VITPAS27, and wood charcoal carrier. In some embodiments, a ratio between the microbial consortium and the wood charcoal carrier is of 1:2. All the strains are equally mixed to achieve a stock microbial consortium of 6×10^8 CFUs and mixed along with the wood charcoal carrier to achieve a moist-powdery formulation. That is, one part of the microbial consortium is mixed with two part of the wood charcoal [weight by weight (W/w)] and directly applied as root treatment to the plants.

[0045] In some embodiments, the bio-fungicide composition is formulated into a seed coating composition that includes 6×10^8 CFUs of the microbial consortium including 1×10^8 CFUs of Streptomyces strain VITPAS3; 1×10^8 CFUs of Micrococcus strain VITPAS7; 1×10^8 CFUs of Streptomyces strain; 1×10^8 CFUs of Actinomyces strain VITPAS16; 1×10^8 CFUs of Rhodococcus strain VITPAS22; and 1×10^8 CFUs of Nesterenkonia strain VITPAS27, and 3% weight by volume (W/v) of sodium alginate cross linked with calcium chloride ($CaCl_2$). All the strains are equally mixed to achieve a stock microbial consortium of 6×10^8 CFUs and mixed along with 3% W/v of sodium alginate cross linked with $CaCl_2$. This seed coating

composition is mixed along with the seeds and dried to form the seeds with the microbial consortium coating to protect the seeds from fungal infection. The microbial consortium coated seeds may be directly sowed in to the soil.

[0046] When the bio-fungicide composition comes in contact with the infected or non-infected seeds, plants or plant parts, the bio-fungicide composition controls the growth of plant pathogens including bacteria and fungi and prevents the plant pathogens from spreading from one plant to another. In some embodiments, the bio-fungicide composition provides an anti-fungal activity and inhibits plant pathogenic fungi when contacting with seeds, plants or plant parts. The plant pathogenic fungi may include *Alternaria solani*, *Rhizoctonia solani*, *Macrophomina phaseolina solani*, *Cochliobolus miyabeanus*, and *Fusarium oxysporum.* The fungi, *Alternaria solani* belongs to the Pleosporaceae family, *Rhizoctonia solani* belongs to the Ceratobasidiaceae family, *Macrophomina phaseolina solani* belongs to the Botryosphaeriaceae family, *Cochliobolus miyabeanus* belongs to the Pleosporaceae family, and *Fusarium oxysporum* belongs to the Nectriaceae family.

[0047] In some embodiments, when the bio-fungicide composition comes in contact with the seeds, plants or plant parts, the bio-fungicide composition provides the plant growth-promoting properties to promote the growth of the plants. The bio-fungicide composition may help in nitrogen fixation, phosphate solubilisation, potassium solubilization, Indole acetic acid (IAA) production, zinc solubilisation, and siderophore production. Thereby, increasing the growth and yield of plants.

[0048] The newly isolated actinobacterial strains in the microbial consortium of the bio-fungicide composition produce one or more substances such as antifungal agents, antibacterial agents, and plant growth-promoting substances, which synergistically prevent the plants or seeds from pathogenic infections caused by the fungal pathogens and promote the growth of plants.

[0049] The plants may include, but not limited to, short-term cultivation crops, broad cultivation crops, horticulture crops, fibre crops, and widely consumed crops such as mung beans, paddy, and tomato plants. The pH of the bio-fungicide composition may be 4-9. The shelf-life of the bio-fungicide composition is about 18 months stored at 4°C. The stock foliar bio-fungicide includes 10% V/v of glycerol containing 1×10^8 CFUs of Streptomyces strain VITPAS3; 1×10^8 CFUs of Micrococcus strain VITPAS7; 1×10^8 CFUs of Streptomyces strain; 1×10^8 CFUs of Actinomyces strain VITPAS16; 1×10^8 CFUs of Rhodococcus strain VITPAS22; and 1×10^8 CFUs of Nesterenkonia strain VITPAS27 resulting in a total microbial consortium concentration of 6×10^8 CFUs. From the stock microbial consortium (6×10^8 CFUs), 6 ml is diluted in 1000 mL of water which produces 1×10^8 CFUs as application dosage. The microbial consortium may be applied to the plants only if fungal infection persists and based on the disease symptoms and progression for every 15 days once.

[0050] The bio-fungicide composition may have storage stability and is storable at ambient condition for a long period of time (upto 18 months) without any degradation in terms of anti-fungal properties, plant growth promoting properties, reduction in microbial

load, changes in pH, ineffectiveness or physical properties such as texture, solubility, and the like.

[0051] FIG. 1 is a flow diagram that illustrates a method of producing a bio-fungicide composition for controlling plant pathogenic fungi and promoting plant growth, according to some embodiments herein. At step 102, newly isolated actinobacterial strains including Streptomyces strain VITPAS3 having a partial nucleotide sequence of SEQ ID NO: 1, Micrococcus strain VITPAS7 having a partial nucleotide sequence of SEQ ID NO: 2, Streptomyces strain VITPAS12 having a partial nucleotide sequence of SEQ ID NO: 3, Actinomyces strain VITPAS16 having a partial nucleotide sequence of SEQ ID NO: 4, Rhodococcus strain VITPAS22 having a partial nucleotide sequence of SEQ ID NO: 5, and Nesterenkonia strain VITPAS27 having a partial nucleotide sequence of SEQ ID NO: 6 are provided. The newly isolated actinobacterial strains are isolated from a marine sediment sample. Each strain is cultured using specific media formulation using the starch casein agar media supplemented with seawater salts and 1–21% NaCl to support the growth of the actinobacterial strains. The newly isolated actinobacterial strains are halo-tolerant.

[0052] At step 104, the newly isolated actinobacterial strains are grown individually by inoculating each strain in ISP-1 (International Streptomyces project -1) media until each strain reaches the concentration of 1×10^8 colony forming units (CFUs) to obtain cultures of each newly isolated actinobacterial strain. The newly isolated actinobacterial strains may be in the form of pure and viable spores, mycelium or hyphae, or smooth mucoid colonies.

[0053] At step 106, a microbial consortium of newly isolated marine actinobacterial strains is obtained by blending the cultures of each newly isolated actinobacterial strain together equally. The viable cell concentration of the stock microbial consortium is 6×10^8 CFUs. At step 108, 1-30% by weight of at least one agriculturally acceptable formulating agent is added with the microbial consortium of the newly isolated marine actinobacterial strains to obtain the bio-fungicide composition. In some embodiments, the at least one agriculturally acceptable formulating agent is a carrier that is wood charcoal. In some embodiments, the at least one agriculturally acceptable formulating agent is an adjuvant that is 10% V/v of glycerol. In some embodiments, the at least one agriculturally acceptable formulating agent is an excipient that is 3% W/v of sodium alginate cross linked with $CaCl_2$.

[0054] In some embodiments, the bio-fungicide composition provides a synergetic anti-fungal activity to control the plant pathogenic fungi and plant growth-promoting properties when contacting with seeds, plants or plant parts.

[0055] FIG. 2 is an exemplary view that illustrates newly isolated actinobacterial strains that are isolated from marine sediment samples, according to some embodiments herein. All the newly isolated actinobacterial strains used for producing a bio-fungicide composition are isolated from the marine sediment samples collected from the Vhan island of Tamil Nadu, India. The newly isolated actinobacterial strains are confirmed using 16S rRNA gene sequence analysis and gene sequence information of the actinobacterial strains is deposited in the National Centre for Biotechnology Information (NCBI) database.

[0056] Plate 202 depicts isolated actinobacterial strain VITPAS3. The strain VITPAS3 is identified as Streptomyces species and has closest sequence similarity with *Streptomyces albus*. The strain VITPAS3 includes a gene sequence encoding 16S ribosomal RNA (rRNA) that includes a nucleotide sequence of SEQ ID NO: 1 and the gene sequence information of the strain VITPAS3 is deposited in NCBI with an accession number: OK036589.1. Plate 204 depicts isolated actinobacterial strain VITPAS7. The strain VITPAS7 is identified as Micrococcus species and has closest sequence similarity with *Micrococcus yunnanensi.* The strain VITPAS7 includes a gene sequence encoding 16S rRNA that includes a nucleotide sequence of SEQ ID NO: 2 and the gene sequence information of the strain VITPAS7 is deposited in NCBI with an accession number: MH041154.1.

[0057] Plate 206 depicts isolated actinobacterial strain VITPAS12. The strain VITPAS12 is identified as Streptomyces species and has closest sequence similarity with *Streptomyces albus*. The strain VITPAS12 includes a gene sequence encoding 16S rRNA that includes a nucleotide sequence of SEQ ID NO: 3 and the gene sequence information of the strain VITPAS12 is deposited in NCBI with an accession number: OK036602.1. Plate 208 depicts isolated actinobacterial strain VITPAS16. The strain VITPAS16 is identified as Actinomyces species and has closest sequence similarity with *Actinomyces oris*. The strain VITPAS16 includes a gene sequence encoding 16S rRNA that includes a nucleotide sequence of SEQ ID NO: 4 and the strain VITPAS16 is deposited in NCBI with an accession number: OK036597.1.

[0058] Plate 210 depicts isolated actinobacterial strain VITPAS22. The strain VITPAS22 is identified as Rhodococcus species and has closest sequence similarity with *Rhodococcus hoagii*strain. The strain VITPAS22 includes a gene sequence encoding 16S rRNA that includes a nucleotide sequence of SEQ ID NO: 5 and the strain VITPAS22 is deposited in NCBI with an accession number: OK036625.1. Plate 212 depicts isolated actinobacterial strain VITPAS27. The strain VITPAS27 is identified as Nesterenkonia species and has closest sequence similarity with *Nesterenkonia alkaliphila.* The strain VITPAS27 includes a gene sequence encoding 16S rRNA that includes a nucleotide sequence of SEQ ID NO: 6 and the strain VITPAS27 is deposited in NCBI with an accession number: OK036630.1.

[0059] All the newly isolated actinobacterial strains are maintained and used in pure or substantially pure form to form the bio-fungicide composition. The newly isolated actinobacterial strains may be used as viable spores or colonies. The isolated actinobacterial strains are cultured using specific media formulation using the starch casein agar media supplemented with seawater salts and 1–21% sodium chloride (NaCl) to support the growth of halo-tolerant strains (or salt-tolerant).

[0060] FIG. 3 is a graphical representation that illustrates antifungal activity of each newly isolated actinobacterial strain on plant pathogenic fungi, according to some embodiments herein. For investigating the antifungal activity, plant fungal pathogens such as *Alternaria solani*, *Rhizoctonia solani*, *Macrophomina phaseolina solani*, *Cochliobolus*

miyabeanus, and *Fusarium oxysporum* are selected and cultured individually using potato dextrose agar (PDA) media. The newly isolated actinobacterial strains (Streptomyces strain VITPAS3, Micrococcus strain VITPAS7, Streptomyces strain VITPAS12, Actinomyces strain VITPAS16, Rhodococcus strain VITPAS22, and Nesterenkonia strain VITPAS27) are grown individually in International Streptomyces's Agar-2 (ISA-2) media that is supplemented with 1-21% NaCl. The newly isolated actinobacterial strains are inoculated as individual spores or pure isolates into the PDA and tested against the individual plant pathogenic fungi. The results on the zone of inhibition are depicted in the graphical representation shown in FIG. 3.

[0061] In the graphical representation, the newly isolated strains are plotted against the X-axis and zone of inhibition is plotted against the Y-axis. From the graphical representation shown in FIG. 3, it is observed that (i) the strain VITPAS3 shows better inhibition of *Alternaria solani* and *Macrophomina phaseolina*, followed by moderate inhibition of *Rhizoctonia solani, Helminthosporium oryzae*, and *Fusarium oxysporum*; (ii) the strain VITPAS7 inhibits *Macrophomina phaseolina* and *Helminthosporium oryzae* better, followed by moderate inhibition of *Rhizoctonia solani, Alternaria solani*, and *Fusarium oxysporum*; (iii) The strain VITPAS12 shows better inhibition of *Macrophomina phaseolina, Fusarium oxysporum*, and *Alternaria solani,* followed by moderate inhibition of *Helminthosporium oryzae*; (iv) The strain VITPAS16 shows better inhibition of *Macrophomina phaseolina* and *Alternaria solani*, followed by moderate inhibition of *Rhizoctonia solani, Helminthosporium oryzae, and Fusarium oxysporum*; (v) The strain VITPAS22 shows better inhibition of *Macrophomina phaseolina*, followed by moderate inhibition of *Alternaria solani, Rhizoctonia solani, and Fusarium oxysporum*, and (iv) the strain VITPAS27 shows the best inhibition of *Alternaria solani,* followed by moderate inhibition of *Fusarium oxysporum and Macrophomina phaseolina.*

[0062] In some exemplary embodiments, the newly isolated actinobacterial strains are tested for strain-compatible nature by cross-streaking individual strains in a single petri plate. It is observed that there is no strain inhibition. Hence, all the newly isolated actinobacterial strains such as Streptomyces strain VITPAS3, Micrococcus strain VITPAS7, Streptomyces strain VITPAS12, Actinomyces strain VITPAS16, Rhodococcus strain VITPAS22, and Nesterenkonia strain VITPAS27 are combined together to form a microbial consortium as shown in FIG. 1.

[0063] FIG. 4 is a graphical representation that illustrates a chromatogram of a microbial consortium that is analyzed using Gas Chromatography and Mass Spectrometry according to some embodiments herein. The microbial consortium is developed from the newly isolated actinobacterial strains and subjected to Gas Chromatography and Mass Spectrometry analysis. The resulting chromatogram is shown as FIG. 4. In the graphical representation, the retention time is plotted against the X-axis and abundance of identified compounds is plotted against the Y-axis. The compounds identified from the Gas □hromatography and Mass Spectrometry analysis are shown in Table 1.

[0064] Table 1

S. No	Retention time	Area (%)	Compounds	Properties
1.	5.973	1.19	Aniline	Herbicide and fungicide
2.	6.660	7.28	Acetamide, N-(2-methylpropyl)-	Fungicide
3.	8.263	0.61	1,1-Dimethyl-1-silacyclobutane	-
4.	8.825	0.74	2,4,6-Cycloheptatrien-1-one	Bactericide, fungicide
5.	10.922	0.61	Acetophenone, 4'-amino-	-
6.	11.056	0.41	Quinoline, 2-methyl-	Antimalarial, antibacterial, antifungal activity
7.	11.509	1.43	Quinazoline, 4-methyl-	Anticancer, antimalarial, antibacterial activity
8.	11.863	0.57	Tryptophol	-
9.	12.885	1.08	2(3H)-Furanone, dihydro-4,4-dimethyl-5-(2-oxopropyl)	Antibacterial
10.	13.170	0.42	3-Buten-1-ol, tert-butyldimethyl	-
11.	14.000	0.53	Azetidine, N-dimethylcarbamoyl	Antibacterial
12.	15.779	2.14	9-Borabicyclo[3.3.1]nonane	-
13.	16.114	0.53	3,3-Diacetyl-2,3,4,5-tetrahydro-2-oxofuran	-
14.	16.651	1.82	1H-Benzo[4,5]furo[3,2-f]indole	-
15.	17.171	0.96	3,5-Heptanedione, 2,6-dimethyl-	-
16.	17.389	5.20	Pyrrolo[1,2-a]pyrazine-1,4-dione	Antibiotic, antioxidative agent
17.	18.413	9.15	Phenol, 3,5-dimethoxy-	Antibacterial, antifungal activity
18.	18.648	0.39	Benzene, 1,3,5-trimethoxy-	-
19.	19.336	0.43	3H-Naphtho[2,3-c]-1,2-dithiol-3-one or 3,4-dichloro-2-methylphenyl trifluoroacetate	-
20.	19.797	14.71	Trimecaine or 3,4-Methylenedioxy-N-propylamphetamine	Local anaesthetic and antiarrhythmic drug or psychedelic drug
21.	20.065	1.05	2,5-Piperazinedione, 3-methyl-6-(phenylmethyl)-	-
22.	20.225	0.39	9-Octadecenoic acid, (E)-	-
23.	20.560	7.24	Perhydro-htx, 1-acetyl-2-depentyl-	-

24.	20.636	1.55	N-Acetyltryptamine	Regulation of sleep and circadian rhythm
25.	20.904	0.73	2-Methyl-1,2,3,4-tetrahydroisoquinoline	-
26.	21.055	1.28	3-Benzyl-6-isopropyl-2,5-piperazinedione	Antibacterial activity
27.	21.265	0.37	1,1-dimethyl-1,2,3,4-tetrahydroisoquinoline	-
28.	21.550	0.45	9H-Carbazole, 2-methyl-	Treating obesity related diseases
29.	22.012	7.15	Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'α,10α)-	Anti inflammatory
30.	23.555	1.640	Bis(2-ethylhexyl) phthalate	-
31.	24.746	2.22	l-Valine, n-propargyloxycarbonyl-, decyl ester	-
32.	25.090	1.86	l-Proline, N-allyloxycarbonyl-, isohexyl ester	-
33.	25.644	21.76	2-Propenoic acid, 2-methyl-, 2-propenyl ester	Antifungal activity

[0065] From FIG. 4 and Table 1, it is clear that the microbial consortium comprises one or more substances such as antifungal agents, and antibacterial agents. These antifungal agents, and antibacterial agents are produced by the newly isolated actinobacterial strains and can prevent pathogenic infections caused by bacterial and fungal pathogens. Thus, the bio-fungicide composition of the present disclosure provides the anti-fungal activity.

[0066] FIGS. 5A-5E are graphical representations that illustrate antifungal activity and Minimum Inhibitory Concentration (MIC) of a microbial consortium of the newly isolated actinobacterial strains on plant pathogenic fungi, according to some embodiments herein. For investigating the antifungal activity and the MIC, cell-free supernatant of the microbial consortium is collected and applied as a foliar application on fungal-infected leaves, flower, fruit, pods, branches, and root of plants, and seed treatment at various concentrations such as 20%, 40%, 60%, 80%, and 100% to control the fungal infections. The results are shown in FIGS. 5A-5E.

[0067] In the graphical representations shown in FIGS. 5A-5E, the concentration percentage (%) of the cell-free supernatant of the microbial consortium is plotted against the X-axis, and % of inhibition of plant pathogenic fungi such as *Alternaria solani*, *Rhizoctonia solani*, *Macrophomina phaseolina solani*, *Fusarium oxysporum,* and *Cochliobolus miyabeanus* is plotted against the Y-axis, respectively. From FIGS 5A-5E, it is observed that 100% of the microbial consortium inhitbits 55% of *Macrophomina phaseolina*, 70%

Fusarium oxysporum, 50% *Helminthosporium oryzae*, 70% *Rhizoctonia solani*, and 40% *Alternaria solani*. Moreover, ~50% of inhibition is observed with 80% of the microbial consortium concentration for *Macrophomina phaseolina*, *Fusarium oxysporum*, and *Rhizoctonia solani*. In view of this, it is concluded that Minimum Inhibitory Concentration required for the microbial consortium to inhibit the plant pathogenic fungi is 80%-100%.

[0068] Moreover, as shown in FIG. 3, none of the strain from Streptomyces strain VITPAS3, Micrococcus strain VITPAS7, Streptomyces strain VITPAS12, Actinomyces strain VITPAS16, Rhodococcus strain VITPAS22, and Nesterenkonia strain VITPAS27 inhibits all the five pathogenic fungi that are investigated. Whereas, when the strains are combined, the microbial consortium inhibits all the five pathogenic fungi as shown in FIGS 5A-5E. From FIG. 3 and FIGS 5A-5E, it is clear that the newly isolated actinobacterial strains in the microbial consortium (or the bio-fungicide composition) synergistically work together to provide the anti-fungal activity to control the plant pathogenic fungi.

[0069] In some exemplary embodiments, plant growth-promoting properties of a microbial consortium-based bio-fungicide composition of the present disclosure are analysed with different plants infected with different fungal pathogens. In such experiments, each selected and infected seeds or plant parts (such as leaves, root, and stem) are treated by foliar spraying or root application or seed coating with different fungicides such as the microbial consortium-based bio-fungicide composition of the present disclosure, Pseudomonas-based bio-fungicide, Bacillus-based bio-fungicide, and chemical fungicide. In the experiments, the plants without infection are considered as control-1 and the infected plants without any fungicide treatment are considered as control-2. The plants are collected for plant growth analysis after a predetermined time period of treatments.

[0070] FIG. 6A is a graphical representation that illustrates shoot length and root length of *Alternaria solani*-infected tomato plants that are treated with different fungicides according to some embodiments herein. In the graphical representation, different fungicide treatments are plotted against the X-axis, and plant growth associated with the shoot length and the root length of plants is plotted against the Y-axis.

[0071] From the graphical representation shown in FIG. 6A, it is inferred that the *A. solani*-infected tomato plant that is treated with the microbial consortium-based bio-fungicide composition shows better results than other fungicides and controls 1 and 2. The microbial consortium-based bio-fungicide composition promotes the growth of the tomato plant in addition to controlling the fungus *A. solani.*

[0072] FIG. 6B is a graphical representation that illustrates shoot length and root length of *Fusarium oxysporum*-infected tomato plants that are treated with different fungicides according to some embodiments herein. In the graphical representation, different fungicide treatments are plotted against the X-axis, and plant growth associated with the shoot length and the root length of plants is plotted against the Y-axis.

[0073] From the graphical representation shown in FIG. 6B, it is inferred that the *F. oxysporum*-infected tomato plant that is treated with the microbial consortium-based bio-

fungicide composition shows better results than other fungicides and control 1 and 2. The microbial consortium-based bio-fungicide composition promotes the growth of the tomato plant in addition to controlling *Fusarium oxysporum* induced wilt.

[0074] FIG. 6C is a graphical representation that illustrates shoot length and root length of *Rhizoctonia solani*-infected paddy plants that are treated with different fungicides according to some embodiments herein. In the graphical representation, different fungicide treatments are plotted against the X-axis, and plant growth associated with the shoot length and the root length of plants is plotted against the Y-axis.

[0075] From the graphical representation shown in FIG. 6C, it is inferred that the microbial consortium-based bio-fungicide composition controls *Rhizoctonia solani* induced root rot infection in paddy plants with a slight exhibition of plant growth promotion compared to other fungicides and control 1 and 2.

[0076] FIG. 6D is a graphical representation that illustrates shoot length and root length of *Helminthosporium oryzae*-infected paddy plants that are treated with different fungicides according to some embodiments herein. In the graphical representation, different fungicide treatments are plotted against the X-axis, and plant growth associated with the shoot length and the root length of plants is plotted against the Y-axis.

[0077] From the graphical representation shown in FIG. 6D, it is inferred that the microbial consortium-based bio-fungicide composition controls *Helminthosporium oryzae* based leaf infection on paddy plants and shows potential plant growth-promoting properties on the paddy plants that other fungicides and controls 1 and 2.

[0078] FIG. 6E is a graphical representation that illustrates shoot length and root length of *Macrophomina phaseolina*-infected green gram plants that are treated with different fungicides according to some embodiments herein. In the graphical representation, different fungicide treatments are plotted against the X-axis, and plant growth associated with the shoot length and the root length of plants is plotted against the Y-axis.

[0079] From the graphical representation shown in FIG. 6E, it is inferred that the microbial consortium-based bio-fungicide composition controls *Macrophomina phaseolina* induced stem canker on the green gram plants and shows potential plant growth-promoting properties on the green gram plants than other fungicides and controls 1 and 2.

[0080] FIG. 6F is a graphical representation that illustrates shoot length and root length of green gram plants that are grown from seed infected with *Rhizoctonia solani* according to some embodiments herein. The seeds of the green gram plants are immersed in different fungicides for 12 hours, and sowed on the pots or, fields or suitable growth media supporting sprouting. After that, the seedlings are analyzed and the results are depicted in FIG. 6F. In the graphical representation, different fungicide treatments are plotted against the X-axis, and plant growth associated with the shoot length and the root length of plants is plotted against the Y-axis.

[0081] From the graphical representation shown in FIG. 6F, it is inferred that the microbial consortium-based bio-fungicide composition reduces the seed susceptibility to the

infection of *Rhizoctonia solani* at the seed sprouting stage and controls the fungal progression on the seeds. Also, the microbial consortium-based bio-fungicide composition shows potential plant growth-promoting properties on the green gram plants than other fungicides and control 1 and 2.

[0082] In view of FIGS. 6A-6E, it is clear that the plants treated with the microbial consortium of the bio-fungicide composition of the present disclosure show better fungal disease susceptibility and plant growth and yield than untreated or uninfected control plants and plants with fungal infection. Hence, the bio-fungicide composition provides plant growth-promoting properties in addition to anti-fungal activity to control the plant pathogenic fungi, when contacting with at least one of seeds, plants or plant parts.

[0083] The foregoing description of the specific embodiments will so fully reveal the general nature of the embodiments herein that others can, by applying current knowledge, readily modify and/or adapt for various applications without departing from the generic concept, and, therefore, such adaptations and modifications should be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. Therefore, while the embodiments herein have been described in terms of preferred embodiments, those skilled in the art will recognize that the embodiments herein can be practiced with modification within the spirit and scope of the appended claims.

CLAIMS

I/We Claim:

1. A bio-fungicide composition for controlling plant pathogenic fungi and promoting plant growth, wherein the bio-fungicide composition comprising:

a microbial consortium of two or more newly isolated marine actinobacterial strains that comprises,

characterized in that,

Streptomyces strain VITPAS3 comprising a gene sequence encoding 16S ribosomal RNA (rRNA) comprising a partial nucleotide sequence of SEQ ID NO: 1;

Micrococcus strain VITPAS7 comprising a gene sequence encoding 16S rRNA comprising a partial nucleotide sequence of SEQ ID NO: 2;

Streptomyces strain VITPAS12 comprising a gene sequence encoding 16S rRNA comprising a partial nucleotide sequence of SEQ ID NO: 3;

Actinomyces strain VITPAS16 comprising a gene sequence encoding 16S rRNA comprising a partial nucleotide sequence of SEQ ID NO: 4;

Rhodococcus strain VITPAS22 comprising a gene sequence encoding 16S rRNA comprising a partial nucleotide sequence of SEQ ID NO: 5; and

Nesterenkonia strain VITPAS27 comprising a gene sequence encoding 16S rRNA comprising a partial nucleotide sequence of SEQ ID NO: 6; wherein the two or more newly isolated marine actinobacterial strains are combined together to form the bio-fungicide composition that provides a synergetic anti-fungal activity to control the plant pathogenic fungi and plant growth-promoting properties when contacting with at least one of seeds, plants or plant parts.

2. The bio-fungicide composition as claimed in claim 1, wherein a concentration of each marine actinobacterial strain in the microbial consortium is 1×10^8 colony forming units (CFUs), wherein a viable stock concentration of the microbial consortium is 6×10^8 CFUs in the bio-fungicide composition.

3. The bio-fungicide composition as claimed in claim 1, further comprising at least one agriculturally acceptable formulating agent that comprises a carrier, an adjuvant, or an excipient, wherein a concentration of at least one agriculturally acceptable formulating agent is in a range between 1-30% by weight.

4. The bio-fungicide composition as claimed in claim 1, wherein the microbial consortium is formulated into at least one form of liquid, liquid concentrates, solid granules, powder, emulsions, paste, semi-solid formations, wettable powders, or seed coatings using the at least one pharmaceutically acceptable formulating agent.

5. The bio-fungicide composition as claimed in claim 1, wherein the bio-fungicide composition is formulated into a foliar spray composition that comprises 6×10^8 CFUs of the microbial consortium including 1×10^8 CFUs of Streptomyces strain VITPAS3; 1×10^8 CFUs of Micrococcus strain VITPAS7; 1×10^8 CFUs of Streptomyces strain; 1×10^8 CFUs of Actinomyces strain VITPAS16; 1×10^8 CFUs of Rhodococcus strain VITPAS22; and 1×10^8 CFUs of Nesterenkonia strain VITPAS27, and 10% volume by volume (V/v) of glycerol, wherein 6 mL of the foliar spray composition is diluted with 1000 mL of water.

6. The bio-fungicide composition as claimed in claim 1, wherein the bio-fungicide composition is formulated into a root fungicide composition that comprises 6×10^8 CFUs of the microbial consortium including 1×10^8 CFUs of Streptomyces strain VITPAS3; 1×10^8 CFUs of Micrococcus strain VITPAS7; 1×10^8 CFUs of Streptomyces strain; 1×10^8 CFUs of Actinomyces strain VITPAS16; 1×10^8 CFUs of Rhodococcus strain VITPAS22; and 1×10^8 CFUs of Nesterenkonia strain VITPAS27, and wood charcoal carrier, wherein a ratio between the microbial consortium and the wood charcoal carrier is of 1:2.

7. The bio-fungicide composition as claimed in claim 1, wherein the bio-fungicide composition is formulated into a seed coating composition that comprises 6×10^8 CFUs of the microbial consortium including 1×10^8 CFUs of Streptomyces strain VITPAS3; 1×10^8 CFUs of Micrococcus strain VITPAS7; 1×10^8 CFUs of Streptomyces strain; 1×10^8 CFUs of Actinomyces strain VITPAS16; 1×10^8 CFUs of Rhodococcus strain VITPAS22; and 1×10^8 CFUs of Nesterenkonia strain VITPAS27, and 3% weight by volume (W/v) of sodium alginate cross linked with calcium chloride ($CaCl_{2)}$).

8. The bio-fungicide composition as claimed in claim 1, wherein the bio-fungicide composition controls the plant pathogenic fungi that comprises *Alternaria solani*, *Rhizoctonia solani*, *Macrophomina phaseolina solani*, *Cochliobolus miyabeanus*, and *Fusarium oxysporum*.

9. A method of producing a bio-fungicide composition for controlling plant pathogenic fungi and promoting plant growth, characterized in that, the method comprising:

providing newly isolated actinobacterial strains including Streptomyces strain VITPAS3 comprising a gene sequence encoding 16S ribosomal RNA (rRNA) that comprises a partial nucleotide sequence of SEQ ID NO: 1, Micrococcus strain VITPAS7 comprising a gene sequence encoding 16S rRNA that comprises a partial nucleotide sequence of SEQ ID NO: 2, Streptomyces strain comprising a gene sequence encoding 16S rRNA that comprises a partial nucleotide sequence of SEQ ID NO: 3, Actinomyces strain VITPAS16 comprising a gene sequence encoding 16S rRNA that comprises a partial nucleotide sequence of SEQ ID NO: 4, Rhodococcus strain VITPAS22 comprising a gene sequence encoding 16S rRNA that comprises a partial nucleotide sequence of SEQ ID NO: 5, and Nesterenkonia strain

VITPAS27 comprising a gene sequence encoding 16S rRNA that comprises a partial nucleotide sequence of SEQ ID NO: 6;

obtaining cultures of each newly isolated actinobacterial strain by inoculating each strain in International Streptomyces project -1 (ISP-1) media to grow until each strain reaches a concentration of 1×10^8 colony forming units (CFUs);

obtaining a microbial consortium of the newly isolated marine actinobacterial strains by evenly blending the cultures of each newly isolated actinobacterial strain together, wherein a viable stock concentration of the microbial consortium is 6×10^8 CFUs; and

adding 1-30% by weight of at least one agriculturally acceptable formulating agent with the microbial consortium of the newly isolated marine actinobacterial strains to obtain the bio-fungicide composition, wherein the bio-fungicide composition provides a synergetic anti-fungal activity to control the plant pathogenic fungi and plant growth-promoting properties when contacting with at least one of seeds, plants or plant parts.

10. The method as claimed in claim 9, wherein the microbial consortium of the newly isolated marine actinobacterial strains is stable at a temperature in a range between 10°C and 45°C, a pH in a range between 4 and 9, and a salt concentration in a range of from 1% to 21% sodium chloride (NaCl), wherein the microbial consortium is storable at 27°C for 9 months and 4°C for 18 months.

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ABSTRACT

ACTINOBACTERIAL STRAINS-BASED BIO-FUNGICIDE COMPOSITION FOR CONTROLLING PLANT PATHOGENIC FUNGI AND METHOD FOR PRODUCING THE SAME

An actinobacterial strains-based bio-fungicide composition for controlling plant pathogenic fungi is provided. The bio-fungicide composition includes a microbial consortium of two or more newly isolated marine actinobacterial strains that includes (i) Streptomyces strain VITPAS3 having a nucleotide sequence of SEQ ID NO: 1; (ii) Micrococcus strain VITPAS7 having nucleotide sequence of SEQ ID NO: 2; (iii) Streptomyces strain VITPAS12 having nucleotide sequence of SEQ ID NO: 3; (iv) Actinomyces strain VITPAS16 having nucleotide sequence of SEQ ID NO: 4; (v) Rhodococcus strain VITPAS22 having nucleotide sequence of SEQ ID NO: 5; and (vi) Nesterenkonia strain VITPAS27 having nucleotide sequence of SEQ ID NO: 5. A concentration of each strain in the microbial consortium is 1×10^8 CFUs. The strains are combined together to form the bio-fungicide composition that provides synergetic anti-fungal activity to control plant pathogenic fungi and plant growth-promoting properties when contacting with seeds, plants or plant parts.

FIG. 1