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(54) Title: A FORMULATION OF BIO-INOCULANTS FOR AGRICULTURE WITH ENHANCED SHELF LIFE

(57) Abstract: The disclosure provides for micro-encapsulated micro-organisms, and methods to produce the same. The micro-encapsulated micro-organisms can be coated on seed surface, which thereby enhances the growth potential of the plant, as micro-organisms colonise plant parts. Live organisms are encapsulated in permeable polymer, which allows permeation of cellular and nutritional contents to the core and enable controlled release of endophytes, and the microencapsulation protects micro-organisms against the toxic effects of pesticides/fungicides. Seeds coated with micro-encapsulated endophytes act as an efficient delivery vehicle for targeted application of microbes.



A FORMULATION OF BIO-INOCULANTS FOR AGRICULTURE WITH ENHANCED SHELF LIFE

5 **FIELD OF INVENTION**

[001] This disclosure relates to microencapsulation of microbial inoculants; Arbuscular *Mycorrhiza* spores and *Rhizobium* inoculants to prepare biofertilizers. The use of these biofertilizers demonstrates plant growth promoting effect.

10 **BACKGROUND OF THE INVENTION**

[002] Microbial inoculant biofertilizers are becoming an effective tool for sustainable agriculture as they reduce the use of chemical fertilizers. Beneficial micro-organisms particularly endophytic plant growth promoting micro-organisms are viable alternatives to chemical fertilizers used in agriculture. Arbuscular *Mycorrhizal* (AM) fungi colonize the root of plants, forming a symbiotic association by feeding on plant roots, and also promote plant growth by stimulating the uptake of phosphorus (Allen and Allen, 1986, Nelsen, 1987). Nitrogen-fixing bacteria, such as *Rhizobium* spp., are symbiotically associated with plant and colonize the roots of legumes to fix atmospheric nitrogen in a usable form to be taken up by plants.

15 [003] Microbial inoculation of seeds is an ideal mechanism for delivery of high densities of beneficial micro-organisms to soil and the rooting medium, where they can colonise emerging plant roots. However, use of microbial inoculants via seed has been limited by technical challenges in maintaining high numbers of functional microorganisms on seed during seed treatment. Early method of producing inoculant involves mixing an active living rhizobium culture directly with such as soil or peat. But the disadvantage of this is low shelf life. Several methods have been employed to coat seeds with micro-organisms. The methods include dusting, pelleting, and film coating. Dusting the seeds with a dormant organism gives rise to a product which has

an excellent shelf life but the dust doesn't adhere completely to the seed. Whenever seeds are handled during sowing they release loose dust to the environment. Film coated seeds have the advantage of not releasing the dust but they incorporate moisture in the process. Bacteria and fungi react with water to form products which are rehydrated during coating process which have poor viability upon subsequent drying.

[004] The most common forms of controlled release materials are coated microcapsules, coated solids including both porous and non-porous particles and coated aggregates of solid particles. In some instances, a water soluble encapsulating film is desired, which releases the encapsulated material when the capsule is placed in contact with water (US Pat. No. 4,956,129). A further interfacial polymerization process is described in U.S. Pat. No 3,726,804 whereby all the film-forming ingredients initially reside in hydrophobic droplets which also contain a low boiling or polar solvent in addition to the material to be encapsulated. Upon heating, the solvent is released into the aqueous phase (the continuous phase of the emulsion), and the film-forming materials accumulate at the interface and polymerize. U.S. Pat. No. 3,111,407 describes a freeze drying method which forms encapsulated droplets at an instant of atomization. These processes vary in terms of equipment expense, energy requirements, ease of controlling the microcapsule size, the need for extra reagents such as catalysts and settling agents, and percent microcapsule phase.

[005] Increased understanding of the interactions between micro-organisms, formulation components and the seed is essential in development of new seed inoculants. The use of encapsulated cells for environmental applications has several advantages over free cell formulations, however they are faced with certain drawbacks such as mechanical stability, controlled release, environmentally responsive, biodegradable, and cost-effectiveness (P Siva kumar et al., *Int.J.Curr.Microbiol.App.Sci* (2014) 3(6) 415-422). It is important to formulate the micro-organisms in an environment where they are in a metabolically and physiologically competent state. The formulation should contain ingredients that will

aid in stabilization and protection of the microbial cells during storage, transport and availability at the target zone.

SUMMARY OF THE INVENTION

5 [006] In an aspect of present disclosure, there is provided a method of encapsulation of at least one micro-organisms, said method comprises the steps of: (a) preparing a slurry comprising at least one protein, and at least one sugar in water by heating the ingredients at a temperature in the range of 60-95°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with a neutralizing agent of said slurry in
10 the range of 5.5-6.5 to obtain slurry II; (c) obtaining at least one micro-organism, selected from a group consisting of *Rhizobium leguminosarum*, *Bradyrhizobium japonicum*, *Mesorhizobium cicero*, Arbuscular Mycorrhizal spores, and combinations thereof; (d) washing said micro-organism with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry
15 II and stirring at 500-1500rpm to obtain a mixture; (f) adding oil to said mixture to obtain an emulsion, wherein said oil to said water v/v ratio is in the range of 1:2-1:3; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with a coating agent to obtain coated beads; (i) mixing said coated beads with aqueous suspension of inorganic salts to obtain hardened microbeads
20 comprising encapsulated micro-organisms.

[007] In an aspect of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method, said method comprising the steps of: (a) preparing a slurry comprising at least one protein, and at least one sugar in water by heating the ingredients at a temperature in the range of 60-95°C; (b) cooling the slurry to room
25 temperature (25-35°C), and adjusting the pH with a neutralizing agent of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining at least one micro-organism, selected from a group consisting of *Rhizobium leguminosarum*, *Bradyrhizobium japonicum*, *Mesorhizobium cicero*, Arbuscular Mycorrhizal spores, and combinations thereof; (d) washing said micro-organism with a buffer such that the pH of micro-

organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding oil to said mixture to obtain an emulsion, wherein said oil to said water v/v ratio is in the range of 1:2-1:3; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with a coating agent to obtain coated beads; (i) mixing said coated beads with aqueous suspension of inorganic salts to obtain hardened microbeads comprising encapsulated micro-organisms.

[008] These and other features, aspects, and advantages of the present subject matter will be better understood with reference to the following description and appended claims. This summary is provided to introduce a selection of concepts in a simplified form. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used to limit the scope of the claimed subject matter.

15 BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

[009] The following drawings form part of the present specification and are included to further illustrate aspects of the present disclosure. The disclosure may be better understood by reference to the drawings in combination with the detailed description of the specific embodiments presented herein.

20 [0010] Figure 1(a, b) outlines the steps involved in encapsulation of micro-organisms, in accordance with an embodiment of present disclosure.

[0011] Figure 2 depicts the survivability of encapsulated micro-organisms in beads coated seed surface vs survivability of naked micro-organisms coated on seed for a period of 12 months, in accordance with an embodiment of present disclosure.

25 [0012] Figure 3(a) depicts the plant growth promoting effect observed in soyabean plant with the use of beads comprising encapsulated micro-organisms (B) vs when no treatment with encapsulated micro-organisms is provided to soyabean seeds (A), in accordance with an embodiment of present disclosure.

[0013] Figure 3(b) depicts the plant growth promoting effect observed in groundnut plant with the use of beads comprising encapsulated micro-organisms (B) vs when no treatment with encapsulated micro-organisms is provided to groundnut seeds(A), in accordance with an embodiment of present disclosure.

5 [0014] Figure 3(c) depicts the plant growth promoting effect observed in green gram plant with the use of beads comprising encapsulated micro-organisms (B) vs when no treatment with encapsulated micro-organisms is provided to green gram seeds(A), in accordance with an embodiment of present disclosure.

10 [0015] Figure 3(d) depicts the plant growth promoting effect observed in blackgram plant with the use of beads comprising encapsulated micro-organisms (B) vs when no treatment with encapsulated micro-organisms is provided to blackgram seeds(A), in accordance with an embodiment of present disclosure.

DETAILED DESCRIPTION OF THE INVENTION

15 [0016] Those skilled in the art will be aware that the present disclosure is subject to variations and modifications other than those specifically described. It is to be understood that the present disclosure includes all such variations and modifications. The disclosure also includes all such steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and
20 all combinations of any or more of such steps or features.

Definitions

[0017] For convenience, before further description of the present disclosure, certain terms employed in the specification, and examples are collected here. These definitions should be read in the light of the remainder of the disclosure and
25 understood as by a person of skill in the art. The terms used herein have the meanings recognized and known to those of skill in the art, however, for convenience and completeness, particular terms and their meanings are set forth below.

[0018] The articles “a”, “an” and “the” are used to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article.

[0019] The terms “comprise” and “comprising” are used in the inclusive, open sense, meaning that additional elements may be included. It is not intended to be construed as “consists of only”.

[0020] Throughout this specification, unless the context requires otherwise the word
5 “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated element or step or group of element or steps but not the exclusion of any other element or step or group of element or steps.

[0021] The term “including” is used to mean “including but not limited to”. “Including” and “including but not limited to” are used interchangeably.

10 [0022] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the disclosure, the preferred methods, and materials are now described. All publications mentioned herein
15 are incorporated herein by reference.

[0023] Microcapsule is a small sphere with a uniform wall around it. The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the wall is sometimes called a shell, coating, or membrane. Most microcapsules have diameters between a few micrometers and a few millimeters.

20 [0024] Microencapsulation is a technique by which solid, liquid or gaseous active ingredients are packaged within a second material for the purpose of shielding the active ingredient from the surrounding environment. Thus the active ingredient is designated as the core material whereas the surrounding material forms the shell. The technique of microencapsulation depends on the physical and chemical properties of
25 the material to be encapsulated. The degradation rate of the microcapsule obtained by the technique of microencapsulation can be fine-tuned by adjusting the molar ratio between the carbohydrates. Varying the carbohydrate content leads to variation in the porosity of the shell. There are three methods for microencapsulation: coacervation (precipitation), spray drying, emulsification. The most common route that provides

high encapsulation for heat liable compound is freeze drying. On further drying the solvent rich phase is removed leaving a hollow shell inside. These hollow particles can be filled with active species in the core.

5 [0025] Microcapsules with impermeable walls are used in products where isolation of active substances is needed, followed by appropriate release under defined conditions. The effects achieved with impermeable microcapsules include: separation of reactive components, protection of sensitive substances against environmental effects, reduced volatility of highly volatile substances and toxicity reduction.

10 [0026] Certain microorganism do not survive well in soil and henceforth they cannot colonise the roots or other parts of the seed. Encapsulating micro-organisms is a good alternative allowing micro-organisms to coat on the seed surface. It also provides an advantage enhancing the viability of micro-organisms in soil's harsh conditions. Certain microorganism which can colonise plant by virtue of being applied to the surface of the seeds, can be a lost natural hence colonisation will be low.

15 The objective of present invention is to provide a simple and inexpensive method for producing microcapsules of bio inoculants on the seed with uniform and readily controlled sizes, which are suitable for ready to use condition. The size, shape, chemical properties viz., degradability, biocompatibility and permeability have to be considered while selecting raw materials during the microencapsulation process. The

20 morphology of the polymer depends on the spray drying, phase separation as well as the free energy between the polymer and aqueous rich phases.

[0027] In the present disclosure multi layered coat technique to coat micro-organisms on seeds has been disclosed. The micro-organism dispersed in an aqueous media exposes them to large and abrupt changes in water activity. It is proposed that a liquid

25 carrier within the coat layers provides a suitable microenvironment for the micro-organism, buffering it against the stress of coating conditions. Film coating is a process in which the coating mixture is sprayed onto the seeds and the seeds are then passed rapidly into a drying zone where the water is driven off to leave a film including polymer that cover them.

[0028] In the instant disclosure micro-encapsulation of AM spores and *Rhizobium* spp has been demonstrated which can be used to impregnate seeds. In such a methodology, it is observed that microbes and seeds remain viable and the resulting plants will be well-colonized by the AM spores and *Rhizobium*. This is surprising in view of the fact that treatment of seeds with fungicide and insecticide can cause toxic effect on beneficial microorganisms (*Mycorrhiza*, *Rhizobium*, *Azotobacter*, *Azospirillum* etc.) during seed treatment. Although formulations such as powder formulations may be applied directly to seed surface, they require greater drying period and are poorly adhesive. The present disclosure describes preparation of an inoculant with fungicide and insecticide without generation of excessive dust. This efficient multilayer coating with dormant living organisms mixed with aqueous solution containing carbohydrate minimizes the loss of viable cells during drying process. And the formulation includes: an active ingredient, endophytic bioinoculants (*Myhorriza* and *Rhizobium*), seed and any additional ingredients (e.g. protective compounds, desiccants, binders, coatings). The basic functions of formulation are to: (1) stabilise the microorganism, (2) aid in its delivery, (3) protect the microbes till their release in to the target zone, (4) enhance the functionality of microbe.

[0029] The disclosure provides for methods for colonisation of plants and seeds with endophytes. The disclosure also provides microcapsules containing highly ordered structures of other bioactive agents, or microorganisms, which are capable of being transported intact through the seed for release at a desired site of action. The method of the present disclosure may be practiced without taking special precautions to prevent seed contamination. However, it is preferred that contamination can be controlled by fungicides or other conventional seed treatments applied, for example, via coating, pelleting, in order to prevent seed or plantlet damage.

[0030] In the instant disclosure viable micro-organisms are encapsulated using organic and inorganic material. These encapsulated micro-organisms and its performance of release studies are done in sterilized soil and coco peat. It is essential that that seed

processing and application treatment (involving the use of encapsulated micro-organisms) do not diminish performance or storability of the seeds.

[0031] The present disclosure is not to be limited in scope by the specific embodiments described herein, which are intended for the purposes of exemplification only. Functionally-equivalent products, compositions, and methods are clearly within the scope of the disclosure, as described herein.

[0032] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism, said method comprises the steps of: (a) preparing a slurry comprising at least one protein, and at least one sugar in water by heating the ingredients at a temperature in the range of 60-95 °C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with a neutralizing agent of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining at least one micro-organism, selected from a group consisting of *Rhizobium leguminosarum*, *Bradyrhizobium japonicum*, *Mesorhizobium cicero*, Arbuscular Mycorrhizal spores, and combinations thereof; (d) washing said micro-organism with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500 rpm to obtain a mixture; (f) adding oil to said mixture to obtain an emulsion, wherein said oil to said water v/v ratio is in the range of 1:2-1:3; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with a coating agent to obtain coated beads; (i) mixing said coated beads with aqueous suspension of inorganic salts to obtain hardened microbeads comprising encapsulated micro-organisms.

[0033] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said method further comprises of drying said hardened microbeads to obtain beads.

[0034] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least one sugar is selected from the group comprising sucrose, lactose, trehalose, starch,

dextrin, and cellulose wherein said at least one sugar weight/volume concentration with respect to said emulsion is in the range of 0.5-6.66%.

[0035] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
5 one sugar is selected from the group comprising sucrose, lactose, trehalose, starch, dextrin, and cellulose wherein said at least one sugar weight/volume concentration with respect to said water is in the range of 2-20%.

[0036] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
10 one sugar is lactose having weight/volume concentration with respect to said emulsion is 3.33%.

[0037] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
15 one sugar is lactose having weight/volume concentration with respect to said water is 10%.

[0038] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least one sugar is lactose having weight/volume concentration with respect to said emulsion is 1.66%.

[0039] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
20 one sugar is lactose having weight/volume concentration with respect to said water is 5%.

[0040] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
25 one sugar is lactose having weight/volume concentration with respect to said emulsion is 5%.

[0041] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least

one sugar is lactose having weight/volume concentration with respect to said water is 15%.

[0042] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
5 one sugar is sucrose having weight/volume concentration with respect to said emulsion is 1.66%.

[0043] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
10 one sugar is sucrose having weight/volume concentration with respect to said water is 5%.

[0044] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
one sugar is sucrose having weight/volume concentration with respect to said emulsion is 5%.

15 [0045] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
one sugar is sucrose having weight/volume concentration with respect to said water is 15%.

[0046] In an embodiment of present disclosure, there is provided a method of
20 encapsulation of at least one micro-organism as described herein, wherein said at least
one sugar is sucrose having weight/volume concentration with respect to said emulsion is 3.33%.

[0047] In an embodiment of present disclosure, there is provided a method of
25 encapsulation of at least one micro-organism as described herein, wherein said at least
one sugar is sucrose having weight/volume concentration with respect to said water is 10%.

[0048] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, at least one protein
weight/volume concentration with respect to said emulsion is present in the range of

0.5-3.33%, said at least one protein is selected from a group comprising sodium caesinate, casein, and whey isolate.

[0049] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, at least one protein
5 weight/volume concentration with respect to said water is present in the range of 2-10%, said at least one protein is selected from a group comprising sodium caesinate, casein, and whey isolate.

[0050] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
10 one protein is casein having weight/volume concentration with respect to said water is 10%.

[0051] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
15 one protein is casein having weight/volume concentration with respect to said water is 3%.

[0052] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
one protein is casein having weight/volume concentration with respect to said water is 2%.

[0053] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
20 one protein is casein having weight/volume concentration with respect to said emulsion is 0.66%.

[0054] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
25 one protein is casein having weight/volume concentration with respect to said emulsion is 1%.

[0055] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least

one protein is casein having weight/volume concentration with respect to said emulsion is 3.33%.

[0056] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
5 one protein is sodium caseinate having weight/volume concentration with respect to said emulsion is 0.66%.

[0057] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said coating agent is selected from group consisting of xanthan gum, gellan, arabic gum, said
10 coating agent weight/volume concentration with respect to emulsion is in the range of 0.025-3.33%.

[0058] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least one coating agent is gellam gum having weight/volume concentration with respect to
15 said emulsion is 0.08%.

[0059] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least one coating agent is xantham gum having weight/volume concentration with respect to said emulsion is 0.03%.

[0060] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least one coating agent is xantham gum having weight/volume concentration with respect to said emulsion is 0.16%.

[0061] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
25 one coating agent is xantham gum having weight/volume concentration with respect to said water is 0.5%.

[0062] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least

one coating agent is xantham gum having weight/volume concentration with respect to said water is 0.1%.

[0063] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said at least
5 one coating agent is gellam gum having weight/volume concentration with respect to said water is 0.24%.

[0064] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, said oil is selected from the group consisting of castor oil, sesame oil, sunflower oil, ginger oil, groundnut
10 oil, palm oil, cashew oil, coconut oil, vegetable oil, and combinations thereof.

[0065] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said inorganic salts is selected from the group consisting of sodium chloride, sodium sulphate, magnesium chloride, magnesium sulphate, calcium chloride, calcium
15 sulphate, and combinations thereof.

[0066] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said molarity of inorganic salts is 0.1-0.3M.

[0067] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said buffer
20 has a pH in the range of 5.5-7.

[0068] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said buffer has a pH in the range of 5.5-6.5.

[0069] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said buffer
25 has a pH in the range of 6-7.

[0070] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said buffer is phosphate buffer.

5 [0071] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein size of Arbuscular Mycorrhiza spores varies from 20-100 μ m.

[0072] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said Arbuscular Mycorrhiza spores is present at about 400/gm of microbead.

10 [0073] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism as described herein, wherein said microbead has a particle size less than 200 μ m.

[0074] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism, said method comprises the steps of: (a) preparing a slurry comprising 10 gm casein, 10gm lactose in 100ml water by heating the ingredients at a temperature in the range of 85 °C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining at least one micro-organism, selected from a group consisting of *Rhizobium leguminosarum*, *Bradyrhizobium japonicum*,
15 *Mesorhizobium ciceri*, Arbuscular Mycorrhizal spores, and combinations thereof; (d) washing said micro-organism with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to
20 obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of sodium chloride to obtain hardened microbeads comprising encapsulated micro-organisms.

[0075] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism, said method comprises the steps of: (a)

preparing a slurry comprising 10 gm casein, 15gm lactose in 100ml water by heating the ingredients at a temperature in the range of 85 °C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of sodium chloride to obtain hardened microbeads comprising encapsulated micro-organism.

[0076] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 10gm lactose in 100ml water by heating the ingredients at a temperature in the range of 85°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of sodium chloride to obtain hardened microbeads comprising encapsulated micro-organism.

[0077] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 10gm lactose in 100ml water by heating the ingredients at a temperature of 85°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to

obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of sodium chloride to obtain hardened microbeads comprising encapsulated micro-organism.

[0078] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 5gm lactose in 100ml water by heating the ingredients at a temperature of 85°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of sodium chloride to obtain hardened microbeads comprising encapsulated micro-organism.

[0079] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 5gm lactose in 100ml water by heating the ingredients at a temperature of 85°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain

a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.5% xantham gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of magnesium sulphate to obtain hardened
5 microbeads comprising encapsulated micro-organism.

[0080] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 20gm sucrose in 100ml water by heating the ingredients at a temperature of 85°C; (b) cooling the slurry to room temperature
10 (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g)
15 stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.5% xantham gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of calcium sulphate to obtain hardened microbeads comprising encapsulated micro-organism.

[0081] In an embodiment of present disclosure, there is provided a method of
20 encapsulation of at least one micro-organism, said method comprises the steps of: (a) preparing a slurry comprising 3 gm casein, 20gm sucrose in 100ml water by heating the ingredients at a temperature of 85°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said
25 spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said

coated beads with 0.1-0.3M solution of sodium chloride to obtain hardened microbeads comprising encapsulated micro-organism.

[0082] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism, said method comprises the steps of: (a) preparing a slurry comprising 3 gm casein, 10gm lactose in 100ml water by heating the ingredients at a temperature of 85°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of magnesium sulphate to obtain hardened microbeads comprising encapsulated micro-organism.

[0083] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 20gm sucrose in 100ml water by heating the ingredients at a temperature of 70°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500 rpm to obtain a mixture; (f) adding 100ml of sesame oil, and 100ml groundnut oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.5% xantham gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of magnesium sulphate to obtain hardened microbeads comprising encapsulated micro-organism.

[0084] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 15gm sucrose in 100ml water by heating the ingredients at a temperature of 70°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 100ml of sesame oil, and 100ml groundnut oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.1% xantham gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of calcium sulphate to obtain hardened microbeads comprising encapsulated micro-organism.

[0085] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism, said method comprises the steps of: (a) preparing a slurry comprising 2 gm sodium caesinate, 15gm lactose in 100ml water by heating the ingredients at a temperature of 70°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.1% xantham gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of magnesium chloride to obtain hardened microbeads comprising encapsulated micro-organism.

[0086] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 10gm lactose in 100ml water by heating the

ingredients at a temperature of 70°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 100ml of sesame oil, and 100ml groundnut oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.5% xanthan gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of calcium chloride to obtain hardened microbeads comprising encapsulated micro-organism.

[0087] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 10gm sucrose in 100ml water by heating the ingredients at a temperature of 63°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining *Rhizobium leguminosarum*; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 100ml of sesame oil, and 100ml groundnut oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellan gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of sodium chloride to obtain hardened microbeads comprising encapsulated micro-organism.

[0088] In an embodiment of present disclosure, there is provided a method of encapsulation of at least one micro-organism, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 10gm lactose in 100ml water by heating the ingredients at a temperature of 70°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining *Rhizobium leguminosarum*; (d) washing said spores with a buffer such that

the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 100ml of sesame oil, and 100ml groundnut oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of sodiumchloride to obtain hardened microbeads comprising encapsulated micro-organism.

[0089] In an embodiment of present disclosure, there is provided a microbead obtained by a method, said method comprising (a) preparing a slurry comprising at least one protein, and at least one sugar in water by heating the ingredients at a temperature in the range of 60-95 °C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with a neutralizing agent of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining at least one micro-organism, selected from a group consisting of *Rhizobium leguminosarum*, *Bradyrhizobium japonicum*, *Mesorhizobium cicero*, Arbuscular Mycorrhizal spores, and combinations thereof; (d) washing said micro-organism with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding oil to said mixture to obtain an emulsion, wherein said oil to said water v/v ratio is in the range of 1:2-1:3; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with a coating agent to obtain coated beads; (i) mixing said coated beads with aqueous suspension of inorganic salts to obtain hardened microbeads comprising encapsulated micro-organisms.

[0090] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method as described herein, wherein said method further comprises of drying said hardened microbead to obtain beads.

[0091] In an embodiment of present disclosure, there is provided a microbead obtained by a method, said method comprising (a) preparing a slurry comprising at least one protein, and at least one sugar in water by heating the ingredients at a temperature in

the range of 60-95 °C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with a neutralizing agent of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining at least one micro-organism, selected from a group consisting of *Rhizobium leguminosarum*, *Bradyrhizobium japonicum*, *Mesorhizobium*
5 *ciceri*, Arbuscular Mycorrhizal spores, and combinations thereof; (d) washing said micro-organism with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding oil to said mixture to obtain an emulsion, wherein said oil to said water v/v ratio is in the range of 1:2-1:3; (g) stirring said
10 emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with a coating agent to obtain coated beads; (i) mixing said coated beads with aqueous suspension of inorganic salts to obtain hardened microbeads; and (j) drying said hardened microbead to obtain beads comprising encapsulated micro-organisms.

[0092] In an embodiment of present disclosure, there is provided an encapsulated
15 micro-organism(s) obtained by a method as described herein, wherein said at least one sugar is selected from the group comprising sucrose, lactose, trehalose, starch, dextrin, and cellulose wherein said at least one sugar weight/volume concentration with respect to said emulsion is in the range of 0.5-6.66%.

[0093] In an embodiment of present disclosure, there is provided an encapsulated
20 micro-organism(s) obtained by a method as described herein, wherein said at least one sugar is selected from the group comprising sucrose, lactose, trehalose, starch, dextrin, and cellulose wherein said at least one sugar weight/volume concentration with respect to said water is in the range of 2-20%.

[0094] In an embodiment of present disclosure, there is provided an encapsulated
25 micro-organism(s) obtained by a method as described herein, at least one protein weight/volume concentration with respect to said emulsion is present in the range of 0.5-3.33%, said at least one protein is selected from a group comprising sodium caesinate, casein, and whey isolate.

[0095] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method as described herein, wherein said coating agent is selected from group consisting of xanthan gum, gellan, arabic gum, said coating agent weight/volume concentration with respect to emulsion is in the range of 0.025-3.33%.

[0096] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method as described herein, said oil is selected from the group consisting of castor oil, sesame oil, sunflower oil, ginger oil, groundnut oil, palm oil, cashew oil, coconut oil, vegetable oil, and combinations thereof.

[0097] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method as described herein, wherein said inorganic salts is selected from the group consisting of sodium chloride, sodium sulphate, magnesium chloride, magnesium sulphate, calcium chloride, calcium sulphate, and combinations thereof.

[0098] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method as described herein, wherein said molarity of inorganic salts is 0.1-0.3M.

[0099] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method as described herein, wherein said buffer has a pH in the range of 5.5-7.

[00100] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method as described herein, wherein said buffer has a pH in the range of 5.5-6.5.

[00101] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method as described herein, wherein said buffer has a pH in the range of 6-7.

[00102] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method, said method comprises the steps of: (a) preparing a slurry comprising 10 gm casein, 10gm lactose in 100ml water by heating

the ingredients at a temperature of 85°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining at least one micro-organism, selected from a group consisting of *Rhizobium leguminosarum*, *Bradyrhizobium japonicum*, *Mesorhizobium*
5 *ciceri*, Arbuscular Mycorrhizal spores, and combinations thereof; (d) washing said micro-organism with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain
10 beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of calcium chloride to obtain hardened microbeads; and (j) drying said hardened microbeads to obtain beads comprising encapsulated micro-organisms.

[00103] In an embodiment of present disclosure, there is provided an encapsulated
15 micro-organism(s) obtained by a method, said method comprises the steps of: (a) preparing a slurry comprising 10 gm casein, 15gm lactose in 100ml water by heating the ingredients at a temperature of 85 °C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said
20 spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said
25 coated beads with 0.1-0.3M solution of calcium chloride to obtain hardened microbeads; and (j) drying said hardened microbeads to obtain beads comprising encapsulated micro-organisms.

[00104] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method, said method comprises the steps of: (a)

preparing a slurry comprising 2 gm casein, 10gm lactose in 100ml water by heating the ingredients at a temperature in 85°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of calcium sulphate to obtain hardened microbeads; and (j) drying said hardened microbeads to obtain beads comprising encapsulated micro-organisms.

[00105] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 10gm lactose in 100ml water by heating the ingredients at a temperature in the range of 85 °C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of calcium sulphate to obtain hardened microbeads; and (j) drying said hardened microbeads to obtain beads comprising encapsulated micro-organisms.

[00106] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 5gm lactose in 100ml water by heating the

ingredients at a temperature in the range of 85°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of sodium chloride to obtain hardened microbeads; and (j) drying said hardened microbeads to obtain beads comprising encapsulated micro-organisms.

[00107] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 5gm lactose in 100ml water by heating the ingredients at a temperature in the range of 85°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.5% xantham gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of sodium chloride to obtain hardened microbeads; and (j) drying said hardened microbead to obtain beads comprising encapsulated micro-organisms.

[00108] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 20gm sucrose in 100ml water by heating the ingredients at a temperature in the range of 85°C; (b) cooling the slurry to room

temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.5% xantham gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of sodium chloride to obtain hardened microbeads; and (j) drying said hardened microbead to obtain beads comprising encapsulated micro-organisms.

[00109] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method, said method comprises the steps of: (a) preparing a slurry comprising 3 gm casein, 20gm sucrose in 100ml water by heating the ingredients at a temperature in the range of 85°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of calcium chloride to obtain hardened microbeads; and (j) drying said hardened microbead to obtain beads comprising encapsulated micro-organisms.

[00110] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method, said method comprises the steps of: (a) preparing a slurry comprising 3 gm casein, 10gm lactose in 100ml water by heating the ingredients at a temperature in the range of 85°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with gluconic acid of said slurry in the

range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 200ml of vegetable oil to said mixture to
5 obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of calcium chloride to obtain hardened microbeads; and (j) drying said hardened microbead to obtain beads comprising encapsulated micro-organisms.

10 **[00111]** In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 20gm sucrose in 100ml water by heating the ingredients at a temperature in the range of 70°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH of said slurry in the range of 5.5-6.5 to
15 obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 100ml of sesame oil, and 100ml groundnut oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm
20 to obtain beads; (h) coating said beads with 0.5% xantham gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of calcium chloride to obtain hardened microbeads; and (j) drying said hardened microbead to obtain beads comprising encapsulated micro-organisms.

[00112] In an embodiment of present disclosure, there is provided an encapsulated
25 micro-organism(s) obtained by a method, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 15gm sucrose in 100ml water by heating the ingredients at a temperature in the range of 70°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores

with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 100ml of sesame oil, and 100ml groundnut oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.1% xantham gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of calcium chloride to obtain hardened microbeads; and (j) drying said hardened microbead to obtain beads comprising encapsulated micro-organisms.

[00113] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 10gm lactose in 100ml water by heating the ingredients at a temperature in the range of 70°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining Arbuscular Mycorrhizal spores; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 100ml of sesame oil, and 100ml groundnut oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.5% xantham gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of calcium chloride to obtain hardened microbeads; and (j) drying said hardened microbead to obtain beads comprising encapsulated micro-organisms.

[00114] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 10gm lactose in 100ml water by heating the ingredients at a temperature in the range of 63°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining *Rhizobium leguminosarum*; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing

said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 100ml of sesame oil, and 100ml groundnut oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of calcium chloride to obtain hardened microbeads; and (j) drying said hardened microbead to obtain beads comprising encapsulated micro-organisms.

[00115] In an embodiment of present disclosure, there is provided an encapsulated micro-organism(s) obtained by a method, said method comprises the steps of: (a) preparing a slurry comprising 2 gm casein, 10gm lactose in 100ml water by heating the ingredients at a temperature in the range of 70°C; (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH of said slurry in the range of 5.5-6.5 to obtain slurry II; (c) obtaining *Rhizobium leguminosarum*; (d) washing said spores with a buffer such that the pH of micro-organism is in the range of 5.6-6.5; (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture; (f) adding 100ml of sesame oil, and 100ml groundnut oil to said mixture to obtain an emulsion; (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads; (h) coating said beads with 0.25% gellam gum to obtain coated beads; (i) mixing said coated beads with 0.1-0.3M solution of calcium chloride to obtain hardened microbeads; and (j) drying said hardened microbead to obtain beads comprising encapsulated micro-organisms.

[00116] Although the subject matter has been described in considerable detail with reference to certain preferred embodiments thereof, other embodiments are possible.

25 EXAMPLES

[00117] The disclosure will now be illustrated with working examples, which is intended to illustrate the working of disclosure and not intended to take restrictively to imply any limitations on the scope of the present disclosure. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly

understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice of the disclosed methods and compositions, the exemplary methods, devices and materials are described herein. It is to be understood that this disclosure is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary.

Example 1

Materials and methods:

[00118] The method employed for preparation of microencapsulated beads containing micro-organism such as Rhizobium and Arbuscular Mycorrhizal spores varied with respect to the temperature of heating. Variation is temperature allowed to calculate the temperature range workable range for preparation of slurry. Further different combination of carbohydrate, protein, oil, and polysaccharide was also evaluated for preparation of micro-encapsulated micro-organisms to determine if the combination of temperature and ingredient influences formation of microbead.

[00119] Method of preparation: The steps involved in the preparation of encapsulated micro-organisms contained in beads comprised the following: (a) First, a slurry comprising protein, and sugar in water was prepared by (a) heating the ingredients at a temperature in the range of 60-95°C. (b) cooling the slurry to room temperature and setting pH in the range of 5.5-6.5. A person skilled in the art may use any agent for setting pH, however in the present application pH was set using gluconic acid. Citric acid can also be used for setting pH (c) microbial suspension is rinsed with a buffer having a pH in the range of 6.5-7.5. pH of the microbial suspension upon wash with the buffer should be between 5.5-7 (d) microbial suspension having a pH in the range of 5.5-6.5 was added to the slurry to obtain a mixture (e) mixture and oil having a v/v ratio in the range of 1:2-1:3 are mixed to obtain an emulsion and stirred, the continuous slow stirring causes the separation of oil beads from oil phase. Oil that can be used is selected from the group consisting of sunflower oil, canola oil, cashew oil,

palm oil, coconut oil, castor oil (f) post separation of beads and oil phase, the beads are coated with a coating agent selected from group consisting of arabic gum, gellan gum, and xanthan gum, and beads harden following addition of salts selected from the group consisting of sodium chloride, sodium sulphate, magnesium chloride, magnesium sulphate, calcium chloride, calcium sulphate and combinations thereof. The final concentration of salt used for hardening is in the range of 0.1-0.3M. The beads are dried by freeze drying, spray drying and stored until use. Figure 1(a, b) outlines the steps involved in the preparation of microbeads.

[00120] The process listed above outlines the key steps in preparation of encapsulated micro-organisms. Listed below are three different processes used for preparation. In each process different sugar, protein, and coating agent was used for preparation. In case of process 1 a temperature of 60°C was used for the preparation of slurry, in process 2 a temperature of 70°C was used for slurry preparation, and in process 3 a temperature of 83°C was used.

[00121] Process 1: Prepared a slurry of protein and carbohydrate in water and heating at 60°C. The microbe culture was dispersed in the slurry after cooling the slurry to room temperature. Added oil to the slurry and stirred at 200-1000rpm for at least 60 minutes. Bead and oil phase separated with continuous stirring. The beads were collected and washed with water and mixed with gellan gum. Gellan gum is a high molecular weight water soluble polysaccharide gum. In microencapsulation process gellan gum acts as membrane coating agent. After membrane coating, the beads are hardened using inorganic salts preferably calcium chloride, sodium chloride and calcium sulphate. The beads are freeze dried. The concentration of coating agent is in the range of about 0.1% to 10% weight/ volume of water. Table 1-3 lists the various beads prepared by process 1.

Table 1

Microbead A			
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Protein source	caesin	2% in water w/v	0.66% in emulsion w/v
Sugar	lactose	10% in water w/v	3.33% in emulsion w/v
Coating agent	Gellan gum	0.25% in water w/v	0.08% in emulsion w/v
Microbe culture	Rhizobium	2% in water w/v	0.66% in emulsion w/v
water		100ml	
Vegetable oil		200ml	
Sodium chloride		0.3-0.9M in water	0.1-0.3M in emulsion

Table 2

Microbead B			
Protein source	Sodium caesinate	2% in water w/v	0.66% in emulsion w/v
Sugar	sucrose	10% in water w/v	3.33% in emulsion w/v
Coating agent	Gellan gum	0.25% in water w/v	0.08% in emulsion w/v
Microbe culture	Rhizobium	2% in water w/v	0.66% in emulsion w/v
water		100ml	
Vegetable oil		200ml	
Sodium chloride		0.3-0.9M in water	0.1-0.3M in emulsion

Table 3

Microbead C			
Protein source	Sodium caesinate	2% in water w/v	0.66% in emulsion w/v

Sugar	lactose	15% in water w/v	5% in emulsion w/v
Coating agent	Gellan gum	0.25% in water w/v	0.08% in emulsion w/v
Microbe culture	Rhizobium	2% in water w/v	0.66% in emulsion w/v
water		100ml	
Vegetable oil		200ml	
Magnesium chloride		0.3-0.9M in water	0.1-0.3M in emulsion

[00122] Process 2: Prepared a slurry of protein, and sugar and heated to 70°C, the slurry was cooled to room temperature. Disperse the AM fungi spore into the solution. Transferred the solution to vegetable oil containing sesame oil, groundnut oil. Continuously stir the mixture using the magnetic stirrer at 1500 rpm for 2 h to separate the beads. The beads are collected and coated with a coating agent and hardened the beads using inorganic salt solution at 200 rpm. The hardened beads are freeze dried and stored. Table 4-6 lists the various microbeads prepared by process 2.

Table 4

Microbead D			
Protein source	casein	2% in water w/v	0.66% in emulsion w/v
Sugar	lactose	10% in water w/v	5% in emulsion w/v
Coating agent	Gellan gum	0.25% in water w/v	0.08% in emulsion w/v
Coating agent	Xanthan gum	0.5% in water w/v	0.16% in emulsion w/v
Microbe culture	Mycorrhizal spores	2% in water w/v	0.66% in emulsion w/v
water		100ml	

Vegetable oil		200ml Seasame oil:100ml Groundnut oil 100ml	
Calcium chloride		0.3-0.9M in water	0.1-0.3M in emulsion

Table 5

Microbead E			
Protein source	casein	2% in water w/v	0.66% in emulsion w/v
Sugar	sucrose	15% in water w/v	5% in emulsion w/v
Coating agent	Xanthan gum	0.1% in water w/v	0.03% in emulsion w/v
Microbe culture	Mycorrhizal spores	2% in water w/v	0.66% in emulsion w/v
water		100ml	
Vegetable oil		200ml Seasame oil:100ml Groundnut oil 100ml	
Calcium sulphate		0.3-0.9M in water	0.1-0.3M in emulsion

Table 6

Microbead F			
Protein source	casein	2% in water w/v	0.66% in emulsion w/v
Sugar	sucrose	20% in water w/v	6.66% in emulsion w/v

Coating agent	Xanthan gum	0.5% in water w/v	0.16% in emulsion w/v
Microbe culture	Mycorrhizal spores	2% in water w/v	0.66% in emulsion w/v
water		100ml	
Vegetable oil		200ml Sesame oil:100ml Groundnut oil 100ml	
Magnesium sulphate		0.3-0.9M in water	0.1-0.3M in emulsion

Process 3

Prepared a slurry of protein and sugar in water. Made a homogeneous mixture by heating at 83°C and dispersing the AM fungal spore to the cooled slurry. Gluconic acid or its derivatives like calcium gluconate, glucono-delta-lactone, sodium gluconate, and potassium gluconate was added to adjust the pH to 5.5-6.5. Added vegetable oil and stirred at 1000rpm for 2 hours to separate the oil and microbeads. The beads are coated with a coating agent, hardened using inorganic salts, and freeze dried. Table 7-14 lists the microbeads prepared according to process 3

10 Table 7

Microbead G			
Protein source	casein	3% in water w/v	1% in emulsion w/v
Sugar	lactose	10% in water w/v	3.33% in emulsion w/v
Coating agent	gellan gum	0.25% in water w/v	0.08% in emulsion w/v
Acid	Gluconic acid	2.5% in water w/v	0.83% in emulsion w/v
Microbe culture	Mycorrhizal spores	2% in water w/v	0.66% in emulsion w/v

water		100ml	
Vegetable oil		200ml Vegetable oil	
Magnesium sulphate		0.3-0.9M in water	0.1-0.3M in emulsion

Table 8

Microbead H			
Protein source	casein	3% in water w/v	1% in emulsion w/v
Sugar	sucrose	20% in water w/v	6.66% in emulsion w/v
Coating agent	Gellam gum	0.25% in water w/v	0.08% in emulsion w/v
Microbe culture	Mycorrhizal spores	2% in water w/v	0.66% in emulsion w/v
acid	Gluconic acid	2.5% in water w/v	0.83% in emulsion w/v
water		100ml	
Vegetable oil		200ml Vegetable oil	
Sodium chloride		0.3-0.9M in water	0.1-0.3M in emulsion

Table 9

Microbead I			
Protein source	casein	2% in water w/v	0.66% in emulsion w/v
Sugar	sucrose	20% in water w/v	6.66% in emulsion w/v
Coating agent	Xanthan gum	0.5% in water w/v	0.16% in emulsion w/v
Microbe	Mycorrhizal	2% in water w/v	0.66% in emulsion w/v

culture	spores		
acid	Gluconic acid	2.5% in water w/v	0.83% in emulsion w/v
water		100ml	
Vegetable oil		200ml Vegetable oil	
Calcium sulphate		0.3-0.9M in water	0.1-0.3M in emulsion

Table 10

Microbead H			
Protein source	casein	2% in water w/v	0.66% in emulsion w/v
Sugar	lactose	5% in water w/v	1.66% in emulsion w/v
Coating agent	Xanthan gum	0.5% in water w/v	0.16% in emulsion w/v
Coating agent	Gellan gum	0.25% in water w/v	0.08% in emulsion w/v
Microbe culture	Mycorrhizal spores	2% in water w/v	0.66% in emulsion w/v
acid	Gluconic acid	2.5% in water w/v	0.83% in emulsion w/v
water		100ml	
Vegetable oil		200ml Vegetable oil	
Magnesium sulphate		0.3-0.9M in water	0.1-0.3M in emulsion

Table 11

Microbead J			
Protein source	casein	2% in water w/v	0.66% in emulsion w/v

Sugar	lactose	5% in water w/v	6.66% in emulsion w/v
Coating agent	Gellam gum	0.25% in water w/v	0.08% in emulsion w/v
Microbe culture	Mycorrhizal spores	2% in water w/v	0.66% in emulsion w/v
acid	Gluconic acid	2.5% in water w/v	0.83% in emulsion w/v
water		100ml	
Vegetable oil		200ml Vegetable oil	
Sodium chloride		0.3-0.9M in water	0.1-0.3M in emulsion

Table 12

Microbead K			
Protein source	casein	2% in water w/v	1% in emulsion w/v
Sugar	lactose	10% in water w/v	3.33% in emulsion w/v
Coating agent	Gellam gum	0.25% in water w/v	0.08% in emulsion w/v
Microbe culture	Mycorrhizal spores	2% in water w/v	0.66% in emulsion w/v
acid	Gluconic acid	2.5% in water w/v	0.83% in emulsion w/v
water		100ml	
Vegetable oil		200ml Vegetable oil	
Sodium chloride		0.3-0.9M in water	0.1-0.3M in emulsion

Table 13

Microbead L			
Protein source	casein	10% in water w/v	3.33% in emulsion w/v
Sugar	lactose	15% in water w/v	5% in emulsion w/v
Coating agent	Gellam gum	0.25% in water w/v	0.08% in emulsion w/v
Microbe culture	Mycorrhizal spores	2% in water w/v	0.66% in emulsion w/v
acid	Gluconic acid	2.5% in water w/v	0.83% in emulsion w/v
water		100ml	
Vegetable oil		200ml Vegetable oil	
Sodium chloride		0.3-0.9M in water	0.1-0.3M in emulsion

Table 14

Microbead M			
Protein source	casein	10% in water w/v	3.33% in emulsion w/v
Sugar	lactose	10% in water w/v	3.33% in emulsion w/v
Coating agent	Gellam gum	0.25% in water w/v	0.08% in emulsion w/v
Microbe culture	Mycorrhizal spores	2% in water w/v	0.66% in emulsion w/v
acid	Gluconic acid	2.5% in water w/v	0.83% in emulsion w/v
water		100ml	
Vegetable oil		200ml Vegetable oil	
Sodium chloride		0.3-0.9M in water	0.1-0.3M in emulsion

Following the creation of encapsulated micro-organism(s) the amount of microbes encapsulated per gram of the dried bead was found to be about 10^{13} .

[00123] Following the preparation of dried beads an experiment was conducted to evaluate the safety provided to microbes the effect of fungicide and insecticide microbes by encapsulation. The dried beads formed above were blended with a carrier and coated on water soluble carrier. The carrier used is a cellulosic substance. To 50 ml of the carrier (polymer), 500ml of water was added to which 10gm of freeze dried beads was added. The suspension obtained was applied to the surface of seeds. This suspension prepared is sufficient for coating seeds sufficient for sowing in 1 acre of land. This provided for seeds whose surface was coated with microbeads labelled as Seed-A. In a parallel set, seeds were coated with microbes directly and labelled as Seed B. The seed-A set, and seed B set was incubated with fungicides like (Carbendazim (0.2%), mancozeb (0.2%), and chemical insectide (Imidacloprid (0.5%). The survival of the microbes following incubation with the respective fungicide, insectide or combination thereof was determined at 0h, 24h, 48h, and 72 hr post incubation.

[00124] Analysis was also done to determine the survivability of microbes on seed A, and Seed B set for a period of 12 months.

[00125] Determination of plant growth promoting effect: Seeds of groundnut, soyabean, green gram, and black gram were used to evaluate the growth promoting effect provided by coating seeds with microbeads of the instant application. To 1kg of seed, 14 ml of a carrier (polymer, a cellulosic substance) was added to which 0.25g of the microbeads were added to obtain seed set A. To the 1 kg of microbead coated seeds 2gm of fungicide (carbendazim (0.2% final conc), or mancozeb (0.2% final concentration) or 5 ml insectide (imidacloprid, final conc 0.5%) was added. In a parallel study, to 1kg seeds without coating with microbes/ or microbeads 2 gm of fungicide (carbendazim (0.2% final conc), or mancozeb (0.2% final concentration) or 5 ml insectide (imidacloprid, final conc 0.5%) was added. The two seed sets (a) bead

coated and fungicide/insecticide treated seeds (b) seed without microbe coating and fungicide insecticide treated were sown in rooting medium that is coco peat. One year old partially digested peat was treated with calcium nitrate and washed 2-3 times of water to obtain electrical conductivity (EC) of 0.5, and pH of 6.5. The peat was
5 sterilized and used for sowing seeds.

Example 2

Results

10 [00126] All the three different process 1-3 were successful in encapsulation of micro-organisms without affect viability. This indicates that a temperature range of 60-90°C can be used for preparation of microbeads. Further the method is not restricted to the type of protein, sugar, coating agent or inorganic salt for the preparation of beads comprising encapsulated micro-organisms. It is observed from Table 1-14 that sugar in
15 the range of 2-20% with respect to water, protein in the range of 2-10% with respect to water, coating agent in the range of 0.1-10% with respect to water, temperature in the range of 60-90°C can be used for preparation. Further sugars which can be used is selected from the group consisting of but not limited to lactose, sucrose, trehalose, starch, dextrin, cellulose. Proteins compatible for use are casein, sodium caesinate, and
20 whey protein isolate. Coating agent that can be used are xanthan gum, gellam gum, arabic gum.

[00127] Next the protection provided against fungicide and insecticide by coating the microbes was evaluated. Table 15 depicts the results. It was observed that seed set B, coated with the microbes and incubated along with fungicide, insecticide or
25 combinations there of, a complete viability lose of cells post 24 hrs of incubation is seen. No microbe could be detected on the seed surface at 48hrs and 72 hrs of incubation. Thus it may be inferred that fungicide/insecticide have an inhibitory effect on the viability of microbes. In case of seeds coated with the microbeads of the instatnt application and incubated along with fungicide, insecticide or combination there of no

decrease in the number of viable cells was observed. The number of microbes observed per gm of seed after 72hrs of incubation was similar to that observed at 0hr. It is inferred that encapsulating the microbes provides protection to microbes against the inhibitory effects of fungicide/insecticide. Thus microencapsulation of microbes allows for a method to mix microbes with incompatible compounds which may have an inhibitory effect on the survival of microbes. It also provides for a method of coating seeds with beneficial micro-organisms.

Table 15 lists the viability of microbe cfu/gm of seed

Seed A (coated with microbeads)				
	0h	24h	48h	72h
Seed A+ fungicide	10^3	10^3	10^3	10^3
Seed A+ insecticide	10^3	10^3	10^3	10^3
Seed A+ fungicide+insecticide	10^3	10^3	10^3	10^3
Seed B (coated with naked microbes)				
Seed B+ fungicide	10^3	10^3	-	-
Seed B + insecticide	10^3	10^3	-	-
Seed B + fungicide + insecticide	10^3	10^3	-	-

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[00128] The survivability of microbes coated on seeds as microbeads was compared to seeds coated with naked microbes for a period up till 12 months. Seeds coated with microbeads or naked microbes were incubated at room temperature for a period upto 12 months and the viable cell count of microbes on the seeds surface was measured. Figure 2 depicts the results obtained. It is observed that the microbe coated on seed surface as microbeads were viable up till a period of 12 months, and no lose in cell viability was observed. However in the case of seeds coated with non encapsulated microbe, no lose in microbe viability could be detected up till 6 months, however from 7 months and onwards the viability decreased. The decrease in viability was about 6 times by 12th month as compared to the first month. It is inferred that

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microencapsulation of microbes enhances the survivability of microbes under harsh conditions, and provides for improved shelf life.

[00129] The seeds coated with microbeads and treated with fungicide and insecticide were sown and colonization by encapsulated microbe to plant part was evaluated. It was observed that infection with Mycorrhizal fungus to plant part could be detected 15 days post sowing and infection with Rhizobium could be detected 40-60 days post sowing. Hence microencapsulation provides for controlled and targeted release of active ingredient, it also mimic as cell bank, and act as carrier of endophytes (plant growth promoting microbes).

[00130] The plants obtained with seeds coated with microbead and fungicide/insecticide treated was compared with plants obtained with seeds treated only with insecticide and fungicide treated. Figure 3(a-d) depicts the difference observed in the root and shoot of the plants. The roots observed have more lateral branching compared to plants obtained from seeds without microbe treatment (B panel of figure 3(a-d) represents treatment with encapsulated micro-organisms, and panel a is control representing no treatment with micro-organisms). Further the root system was extensively developed as seen for the roots of soyabean plant, green gram, and groundnut. Further treatment of seeds with the microbeads also led to a better shoot development as observed by increased shoot length, increased lateral branching and more leaves.

[00131] Seed coating and seed enhancement technology has enormous potential and has become an essential element of success in the production of many crops. Seed coating implies applying a coat around the seed, from a very thin layer, as in the case of film coating, to a thick layer, which results in a real pellet. This enables to improve the quality of the seeds by making them dust-free or by changing the shape and the weight of the seeds. Several methods can be used to coat the seeds including dusting pelleting and film coating. Dusting the seeds with dry dormant inoculants give rise to a product, which has an excellent shelf life. Conventional seed costing technology has various drawbacks. Direct coating of micro-organisms on seeds subjects microbes to subjected to stress during storage and exposure to chemical fungicide and insecticide.

The method of instant disclosure allows coating of micro-organisms to seed using a layer by layer technique.

[00132] Microencapsulated microorganism containing the carbohydrate to minimize loss of viable cell drying. The microorganisms of the present disclosure may be
5 microencapsulated with a suitable food source, which will enhance the micro-organisms ability to survive within the seed until such time that the germinating seed is capable of providing food to the microorganism. Food source includes, dehydrated culture medium; components of same, such as for example, cysteine and bovine serum albumin; and complex carbohydrate or amino acid sources, such as, for
10 example, milk, starch or yeast extract. The composition used to coat the seeds has a strong effect on the viability of the microbial inoculants coated on the seeds. The carbohydrate compounds useful for enhancing survival after temporary rehydration according to the disclosure include gum, sugars, starches and celluloses particularly gum Arabic, xanthan gum, sucrose, mannitol, maltose, trehalose, dextrin, and dextran.
15 Polymer such as PVP that are acceptable as seed coating adhesion promoting agent (or stickers) cannot improve the survivability of microbes.

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I/We Claim:

- 5 1. A method of encapsulation of at least one micro-organism, said method comprises the steps of:
 - (a) preparing a slurry comprising at least one protein, and at least one sugar in water by heating the ingredients at a temperature in the range of 60-95 °C;
 - (b) cooling the slurry to room temperature (25-35°C), and adjusting the pH with a
 - 10 neutralizing agent of said slurry in the range of 5.5-6.5 to obtain slurry II;
 - (c) obtaining at least one micro-organism, selected from a group consisting of *Rhizobium leguminosarum*, *Bradyrhizobium japonicum*, *Mesorhizobium cicero*, Arbuscular Mycorrhizal spores, and combinations thereof;
 - (d) washing said micro-organism with a buffer such that the pH of micro-
 - 15 organism is in the range of 5.6-6.5
 - (e) dispersing said micro-organisms in said slurry II and stirring at 500-1500rpm to obtain a mixture;
 - (f) adding oil to said mixture to obtain an emulsion, wherein said oil to said water v/v ratio is in the range of 1:2-1:3;
 - 20 (g) stirring said emulsion at a speed in the range of 200-1200 rpm to obtain beads;
 - (h) coating said beads with a coating agent to obtain coated beads;
 - (i) mixing said coated beads with aqueous suspension of inorganic salts to obtain hardened microbeads comprising encapsulated micro-organisms.
2. The method as claimed in claim 1, wherein said method further comprises of drying
- 25 said hardened microbead to obtain beads.
3. The method as claimed in claim 1, wherein said at least one sugar is selected from the group comprising sucrose, lactose, trehalose, starch, dextrin, and cellulose wherein

said at least one sugar weight/volume concentration with respect to said emulsion is in the range of 0.5-6.66%.

4. The method as claimed in claim 1, wherein said at least one protein weight/volume concentration with respect to said emulsion is present in the range of 0.5-3.33%, said protein is selected from a group comprising sodium caesinate, casein, and whey isolate.

5. The method as claimed in claim 1, wherein said coating agent is selected from group consisting of xanthan gum, gellan, arabic gum, said coating agent weight/volume concentration with respect to emulsion is in the range of 0.025-3.33%;

10 6. The method as claimed in claim 1, wherein said oil is selected from the group consisting of castor oil, sesame oil, sunflower oil, ginger oil, groundnut oil, palm oil, cashew oil, coconut oil, vegetable oil, and combinations thereof.

7. The method as claimed in claim 1, wherein said inorganic salts is selected from the group consisting of sodium chloride, sodium sulphate, magnesium chloride, magnesium sulphate, calcium chloride, calcium sulphate, and combinations thereof.

8. The method as claimed in claim 1, wherein said buffer has a pH in the range of 5.5-7.

9. The method as claimed in claim 1, wherein size of Arbuscular Mycorrhiza spores varies from 20-100µm.

20 10. The method as claimed in claim 1, wherein said Arbuscular Mycorrhiza spores is present at about 400/gm of microbead.

11. The method as claimed in claim 11, wherein said microbead has a particle size less than 200µm.

12. An encapsulated micro-organism(s) obtained by the method as claimed in any of the claim 1-11

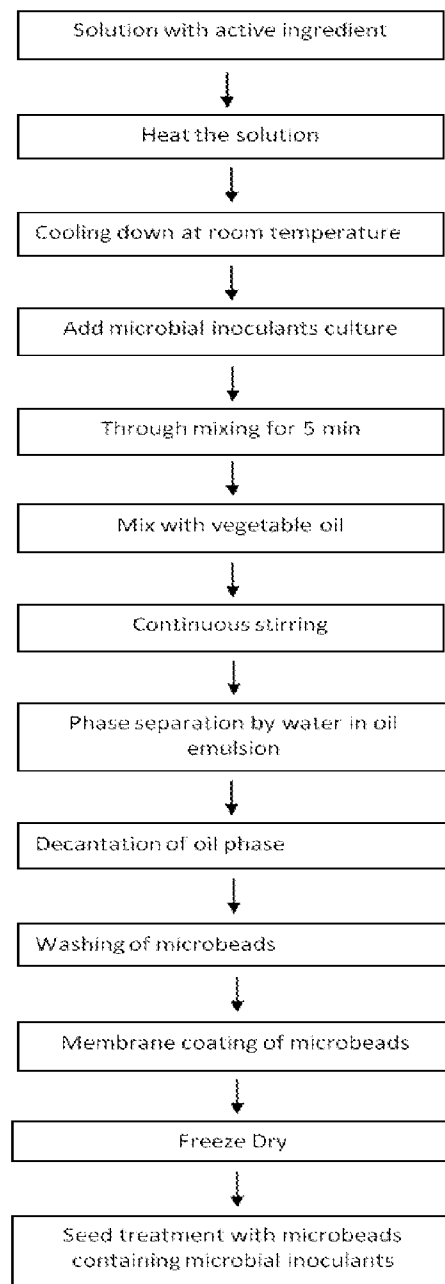


Figure 1(a)

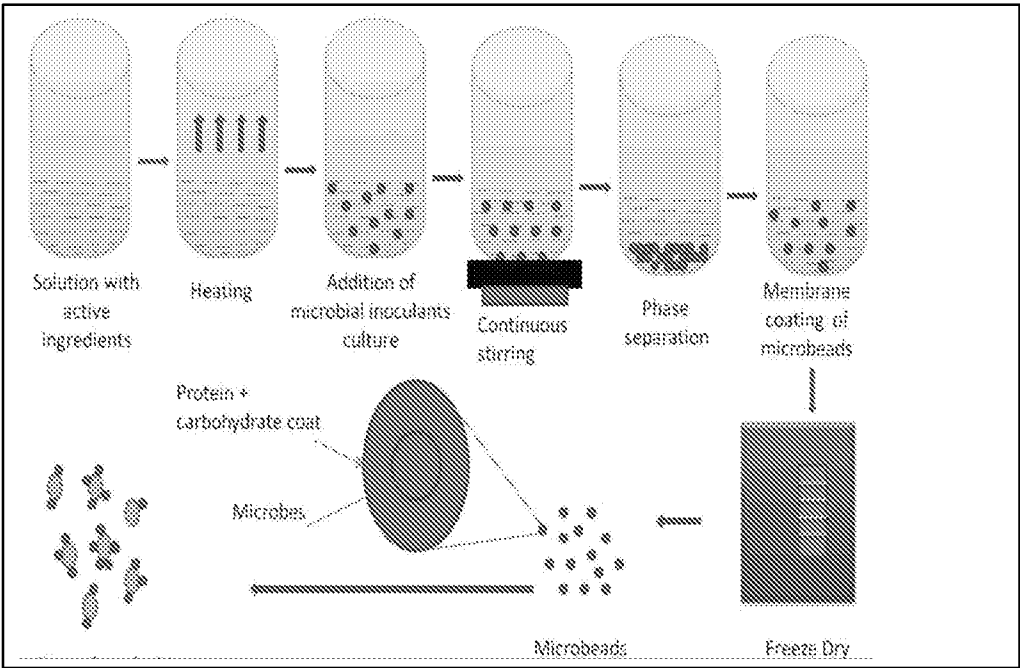


Figure 1(b)

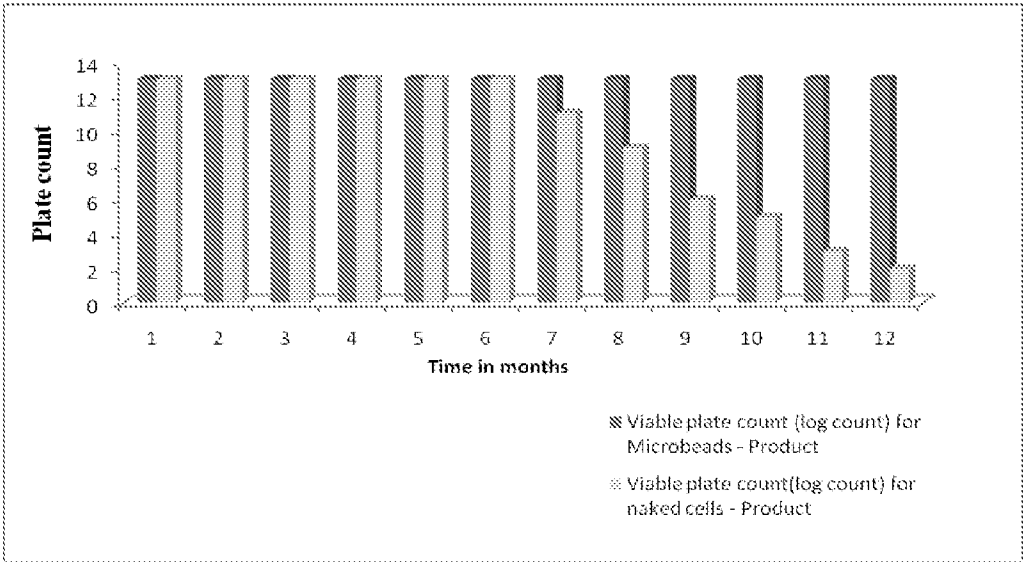


Figure 2

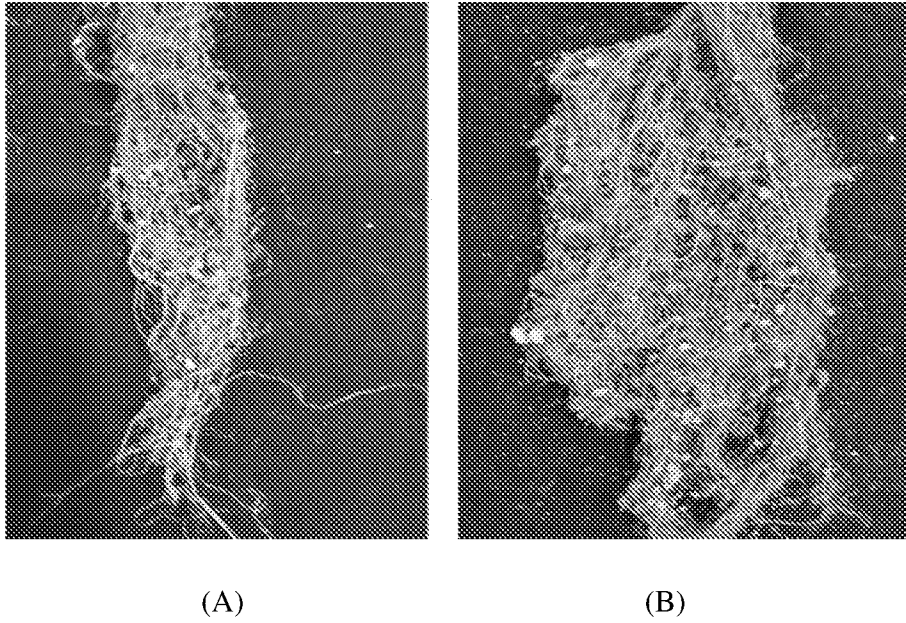


Figure 3(a)

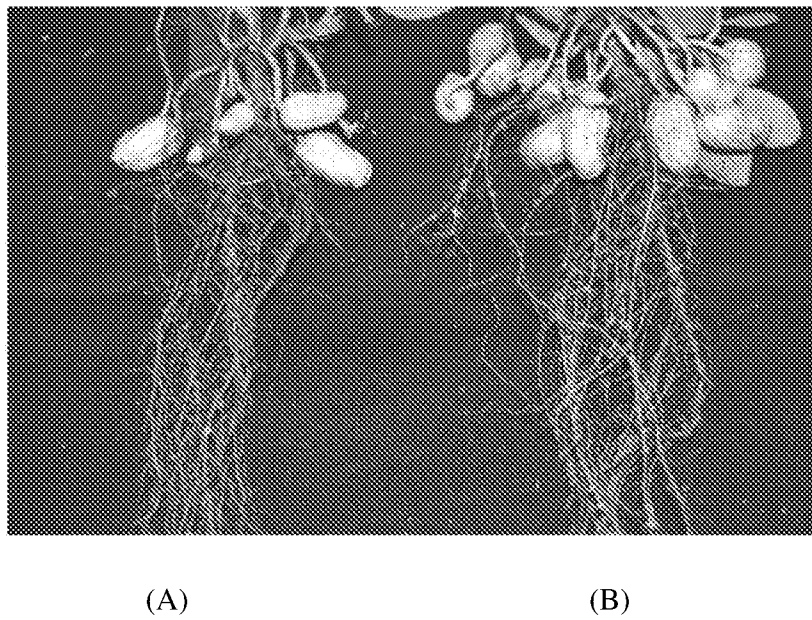


Figure 3(b)

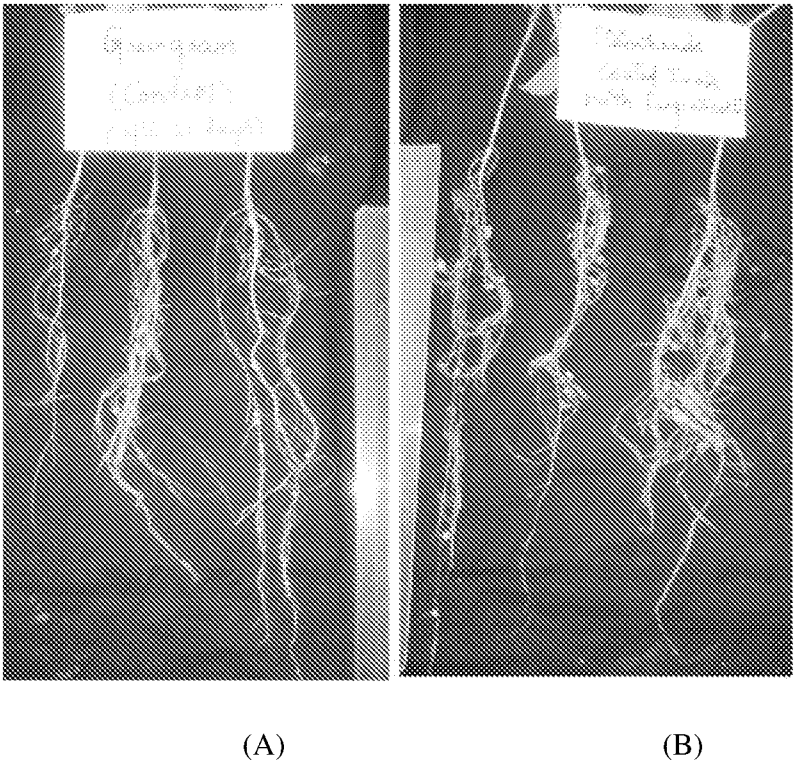


Figure 3(c)

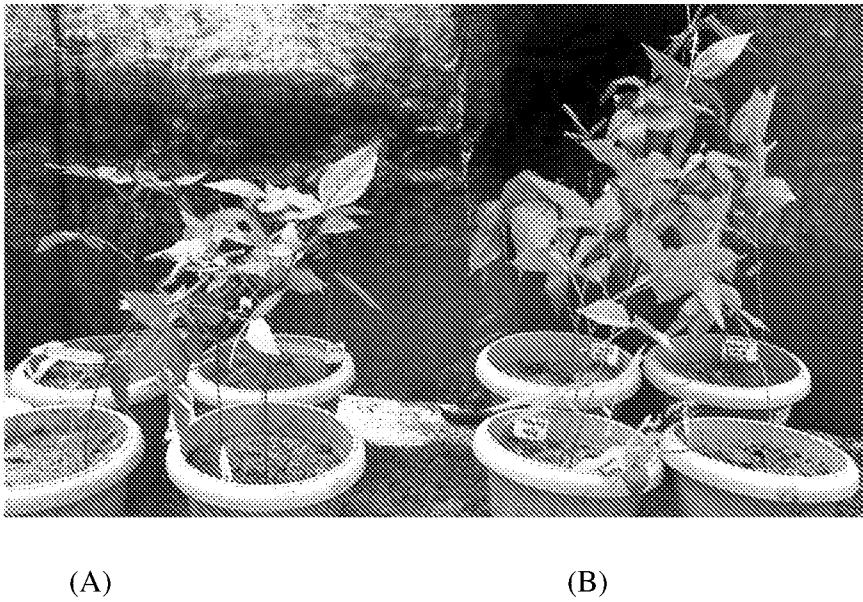


Figure 3(d)

INTERNATIONAL SEARCH REPORT

International application No

PCT/IN2017/050263

A. CLASSIFICATION OF SUBJECT MATTER

INV. A01N63/00

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ROJAN P. JOHN ET AL: "Bio-encapsulation of microbial cells for targeted agricultural delivery", CRC CRITICAL REVIEWS IN BIOTECHNOLOGY, vol. 31, no. 3, 29 September 2010 (2010-09-29), pages 211-226, XP055407352, US ISSN: 0738-8551, DOI: 10.3109/07388551.2010.513327 abstract; table 2 page 212 - page 224; table 1 -----	1-12
X	US 2012/266332 A1 (KUYKENDALL L DAVID [US]) 18 October 2012 (2012-10-18) paragraph [0036] - paragraph [0042]; claims; examples ----- -/--	1-12



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

19 September 2017

Date of mailing of the international search report

26/09/2017

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INTERNATIONAL SEARCH REPORT

International application No

PCT/IN2017/050263

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 755 468 A (JUNG GERARD [FR] ET AL) 5 July 1988 (1988-07-05) column 4, line 53 - column 7, line 50; claims; examples -----	1-12

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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