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UNITED STATES PROVISIONAL PATENT APPLICATION

of

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for

Compositions and Methods for Agri-Inputs and Uses Thereof

Compositions and Methods for Agri-Inputs and Uses Thereof

TECHNICAL FIELD

[0001] The invention relates to plant nutrition and specifically to encapsulation of nutrients.

BACKGROUND

[0002] Agricultural production across the globe is facing unprecedented challenges due to climate change, extreme weather, soil erosion pathogen resistance and environmental pollution. Global population is projected to reach about 10 billion by 2050, thus the demand for food is expected to increase by 60%. The rate of increase of yield and fertilizer (an agri-input) to yield ratio achieved by the green revolution has been declining and detrimental climate change is expected to further limit crop productivity and nutritional quality. To increase yield and improve nutritional quality, plants require balanced nutrition in addition to water and sunlight. However, conventional agri-inputs and their delivery are highly inefficient due to low use efficiency and environmental release. Therefore, more-effective formulations and compositions which can stimulate plant growth, alleviate the negative effect of abiotic/biotic stress, deliver active ingredient efficiently to the plant at the right time in right quantity and release in response to plant's signal are required.

[0003] Biomass-based renewable materials are being explored globally due to their importance for a circular green economy and sustainability. Chitin is the most abundant natural amino polysaccharide. It is present most abundantly in crustaceans, insects, and fungi. Chitin and its derivatives are being considered as a new functional biomaterial of high potential in interdisciplinary applications. For instance, chitosan, a deacetylated derivative of chitin, is being used in industries/sectors such as medicine, textile, food, energy, and water. To date, translational application of chitosan in agriculture is under exploited.

SUMMARY

[0004] In a first aspect, the disclosure provides methods of modifying chitosan for the formulation of chitosan-based nanoparticles.

[0005] In a second aspect, the disclosure provides chitosan-based nanoparticles. The chitosan based nanoparticles may encapsulate nitrogen and/or nitrogen rich compound and/or mixture of compounds such as but not limited to potassium nitrate, calcium nitrate, magnesium nitrate, urea nitrate, urea ammonia nitrate, ammonium nitrate, ammonium sulfate, Urea-hydroxyapatite, amino acids such as arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, valine, glutamine, glutamic acid, glycine, proline, taurine, aspartic acid, serine, and alanine.

[0006] Further aspects and embodiments are provided in the foregoing drawings, detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The following drawings are provided to illustrate certain embodiments described herein. The drawings are merely illustrative, and are not intended to limit the scope of claimed inventions and are not intended to show every potential feature or embodiment of the claimed inventions. The drawings are not necessarily drawn to scale; in some instances, certain elements of the drawing may be enlarged with respect to other elements of the drawing for purposes of illustration.

[0008] Figure 1 is a depiction of Morphological characterizations of the liquid chitosan formulation (TgA-TgE) obtained by Electron Microscopy.

[0009] Figure 2 is a depiction of Morphological characterizations of the liquid chitosan formulation (CTgA-CTgE) obtained 2 by Electron Microscopy.

[00010] Figure 3 is a depiction of Colloidal characterization with reference to hydrodynamic size of the liquid chitosan formulation (TgA- E and CTgA-CTgE) obtained by Dynamic Light Scattering.

[00011] Figure 4 is a depiction of Colloidal characterization with reference to surface zeta potential of the liquid chitosan formulation (TgA- E and CTgA-CTgE) obtained by Dynamic Light Scattering.

[00012] Figure 5 is a table depicting the pH and TKN of Align N Batches 1-7.

[00013] Figure 6 is the UV wavelength analysis of Align N Batches 1-7.

[00014] Figure 7 is the FTIR analysis of Align N 1.

[00015] Figure 8 is the FTIR analysis of Align N 2.

[00016] Figure 9 is the FTIR analysis of Align N 3.

[00017] Figure 10 is the FTIR analysis of Align N 4.

[00018] Figure 11 is the FTIR analysis of Align N 5.

[00019] Figure 12 is the FTIR analysis of Align N 6.

[00020] Figure 13 is the FTIR analysis of Align N 7.

DETAILED DESCRIPTION

[00021] The following description recites various aspects and embodiments of the inventions disclosed herein. No particular embodiment is intended to define the scope of the invention. Rather, the embodiments provide non-limiting examples of various compositions, and methods that are included within the scope of the claimed inventions. The description is to be read from the perspective of one of ordinary skill in the art. Therefore, information that is well known to the ordinarily skilled artisan is not necessarily included.

Definitions

[00022] The following terms and phrases have the meanings indicated below, unless otherwise provided herein. This disclosure may employ other terms and phrases not expressly defined herein. Such other terms and phrases shall have the meanings that they would possess within the context of this disclosure to those of ordinary skill in the art. In some instances, a term or phrase may be defined in the singular or plural. In such instances, it is understood that any term in the singular may include its plural counterpart and vice versa, unless expressly indicated to the contrary.

[00023] As used herein, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. For example, reference to “a substituent” encompasses a single substituent as well as two or more substituents, and the like.

[00024] As used herein, “for example,” “for instance,” “such as,” or “including” are meant to introduce examples that further clarify more general subject matter. Unless otherwise expressly indicated, such examples are provided only as an aid for understanding embodiments illustrated in the present disclosure, and are not meant to be limiting in any fashion. Nor do these phrases indicate any kind of preference for the disclosed embodiment.

[00025] Encapsulation and slow release of nutrients is one method for balancing the nutritional needs of plants. One such nutritional need is nitrogen in the form of amine, nitrate and ammonia. Encapsulation of nitrogen compounds having chemical functional groups as amine, nitrate, ammonia, or any one or all of these such as but not limited to potassium nitrate, calcium nitrate, magnesium nitrate, urea nitrate, urea ammonia nitrate, ammonium nitrate, ammonium sulfate, Urea-hydroxyapatite, amino acids such as arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, valine, glutamine, glutamic acid, glycine, proline, taurine, aspartic acid, serine and alanine is a way of releasing nitrogen in effective amounts for plant growth utilization. There are several benefits of the use of chitosan as a urea-encapsulating/embedding agent. The first benefit is that the chitosan polymer is bio-safe and biodegradable. The chitosan polymer has a biological origin, and has been used for immobilization of enzymes for food, and in biosensing applications. Chitosan can be harvested from fungus, shrimp, crab, lobster, or any combination of these sources. A second benefit compared to inorganic coatings such as sulfur coatings is the absence of cracks. Cracks in the coating cause an immediate release of the coated nutrient upon contact with water. Immediate release of the nutrient defeats the purpose of the encapsulation, which is to slowly release the nutrient. A third benefit of the chitosan polymer coating is the overall health of the soil. The chitosan polymer results in lowered acidification of the soil. Acidification of the soil can be detrimental to specific plant species. Coatings such as sulfur lower the pH of the soil. Additionally, the soil microbes are an important part of soil health. Synthetic polymers cannot be metabolized by soil microbes.

[00026] Embodiments include chitosan-based nanoparticles. In certain embodiments the chitosan-based nanoparticles comprise nitrogen in the form of amine and/or nitrate and/or

ammonia. The nitrogen may be encapsulated by/in the chitosan-based nanoparticles. In particular embodiments, the nitrogen in the form of amine, nitrate, and/or ammonia may be selected potassium nitrate, calcium nitrate, magnesium nitrate, urea nitrate, urea ammonia nitrate, ammonium nitrate, ammonium sulfate, urea-hydroxyapatite, amino acids such as arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, valine, glutamine, glutamic acid, glycine, proline, taurine, aspartic acid, serine, alanine, and combination thereof.

[00027] In certain embodiments, the chitosan-based nanoparticles may comprise at least 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 85, 90, or 95% (w/v) nitrogen. In particular embodiments wherein at least 50, 55, 60, 65, 70, 75, 85, 90, or 95% of the nitrogen is in the chemical form of the amine and/or nitrate and/or ammonia.

[00028] In one embodiment, the encapsulation process begins with the chitosan polymer. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. In certain embodiments the chitosan has a Degree of DeAcetylation (DDA): >80%, MW: <110 KDa. In particular embodiments the DDA is > 85%. The DDA may be 90%. The DDA may be > 95%. In particular embodiments the DDA is about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

[00029] Prior to usage for the formulation of nanoparticles, the chitosan must be dissolved in a solution. Dissolution medium: acidic water having pH ranges between 1 and 5.5, or having a pH < 1, < 2, < 3, < 4, < 5, < 5.5. The acid used may be any acid. In particular embodiments the acid used is one of or a combination of the acids including nitric acid and/or acetic acid and/or hydrochloric acid and/or sulfuric acid. To dissolve the chitosan, the aqueous acidic solution may be kept at temperature between about 68 and about 212 degrees F. In particular embodiments, the temperature is kept at about 68, 72, 82, 92, 101, 112, 122, 132, 142, 152, 162, 172, 182, 192, 202, or 212 degrees F. During the entire reaction period the solution may be kept stirring at rpms between about 50 and about 2500 rpm. After completion of the reaction, typically between 0.3 and 4 hours, the solution was filtered through 200 mesh (74 micron) followed by more than 635 mesh (<20 micron). In the filtered solution, antimicrobial agents such as but not limited to potassium sorbate are added (0.1% v/v). In certain cases, as described below a linker such as sodium tripolyphosphate and/or ammonium sulfate is added in the solution before the addition of antimicrobial agents.

[00030] In one embodiment, the reaction is carried out with specific solutions prepared in accordance with one and/or all of the procedures in Example 1 at the temperature between 68 and 250 degrees F. During the entire reaction period the solution was kept stirring at 400 rpm for uniformity of the substances. After completion of the reaction, typically between 0.3 and 4 hours, the solution was filtered through 200 mesh (74 micron) followed by more than 635 mesh (<20 micron). The nitrogen % in the final solution was maintained between 0.1% and 100%, specifically between 18-24% using the compound and/or mixture of compounds described above.

[00031] In certain embodiments, the chitosan nanoparticles may be formed in the presence of and or loaded with nitrogen in the form of amine and/or nitrate and/or ammonia. The nitrogen may be encapsulated by/in the chitosan-based nanoparticles. In particular embodiments, the nitrogen in the form of amine, nitrate, and/or ammonia may be selected potassium nitrate, calcium nitrate, magnesium nitrate, urea nitrate, urea ammonia nitrate, ammonium nitrate, ammonium sulfate, urea-hydroxyapatite, amino acids such as arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, valine, glutamine, glutamic acid, glycine, proline, taurine, aspartic acid, serine, alanine, and combination thereof.

[00032] > 95% particles were in the spherical, nearly spherical shape or small linear fragments having size between 0.1 nm and 8 microns. (By TEM measurement). 0.1 nm and 10 microns with polydispersity index between 0.01 to 0.99 (by DLS measurement). Surface zeta potential ranged between +5 and 70 mV (measured by DLS). N%: 18-24 Application onto the seed surface, mixing in the soil, exposing root and/or exposing leaves. The application rate ranged between 0.01% to 50% v/w or v/v.

[00033] Embodiments include methods of treating a plant or pant part with any of the compositions described herein. The plant part may be any plant part including, but not limited to a seed, leaf, stem, flower, or root of a plant. Examples species of plant parts include, but are not limited to cereals such as wheat, barley, rye, oat; canola, cotton, eggplant, lettuce, sorghum, soybean, rice, oil seed rape, sugar beet, sugarcane, grapes, lentils, sunflowers, alfalfa, pome fruits; stone fruits; peanuts; coffee; tea; strawberries; turf; vegetables, such as tomatoes, potatoes, cucurbits and lettuce.

[00034] Further embodiments include methods of treating a plant growth medium with any of the compositions described herein. Plant growth medium may comprise one or more of soil,

peat, moss, wood residue, leaf mold, sawdust, bark, bagasse, rice hull, sand, perlite, vermiculite, calcinated clay, polystyrene, urea formaldehyde resins, and hydroponic liquids.

Example 1

Preparation of chitosan using various acids

[00035] [Acetic Acid] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid between 0.3% and 1.9% (v/v) was added. The temperature and agitation was maintained constantly throughout the reaction for up to 4 hours. At the end, when the flakes became invisible to the solution, it was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00036] [Acetic Acid + NaTPP] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid between 0.3% and 1.9% (v/v) was added. When the flakes became invisible to the solution, a solution of sodium tripolyphosphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation was maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00037] [Acetic Acid + Ammonium Sulphate] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were

added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid between 0.3% and 1.9% (v/v) was added. When the flakes became invisible to the solution, a solution of Ammonium Sulphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation was maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00038] [Nitric Acid + Acetic Acid] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid and nitric acid having a ratio of 1:1, 1:2, 2:1 and a total v/v acid strength between 0.3% and 1.9% was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, when the flakes became invisible to the solution, it was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cool down to 77 degree F, and a solution of potassium sorbate was added (1% v/v) and mixed well using agitation.

[00039] [Nitric Acid + Acetic Acid + NaTPP] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid and nitric acid having a ratio of 1:1, 1:2, 2:1 and a total v/v acid strength between 0.3% and 1.9% was added. When the flakes became invisible to the solution, a solution of sodium tripolyphosphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00040] [Nitric Acid + Acetic Acid + Ammonium Sulphate] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid and nitric acid having a ratio of 1:1, 1:2, 2:1 and a total v/v acid strength between 0.3% and 1.9% was added. When the flakes became invisible to the solution, a solution of ammonium sulphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00041] [Nitric Acid + NaTPP] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, nitric acid between 0.3% and 1.9% (v/v) was added. When the flakes became invisible to the solution, a solution of sodium tripolyphosphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation was maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00042] [Nitric Acid + Ammonium sulphate] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, nitric acid between 0.3% and 1.9% (v/v) was added. When the flakes became invisible to the solution, a solution of ammonium sulphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction

for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00043] [Nitric Acid] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, nitric acid between 0.3% and 1.9% (v/v) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, when the flakes became invisible to the solution, it was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 -1% v/v) and mixed well using agitation.

[00044] [Hydrochloric Acid] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, hydrochloric acid between 0.3% and 1.9% (v/v) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, when the flakes became invisible to the solution, it was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 -1% v/v) and mixed well using agitation.

[00045] [Hydrochloric Acid + NaTPP] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, hydrochloric acid between 0.3% and 1.9% (v/v) was added. When the flakes became invisible to the solution, a solution of sodium tripolyphosphate (0.001 - 1% v/v or v/w)

was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00046] [Hydrochloric Acid + Ammonium sulphate] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, hydrochloric acid between 0.3% and 1.9% (v/v) was added. When the flakes became invisible to the solution, a solution of ammonium sulphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00047] [Sulfuric Acid] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, sulfuric acid between 0.3% and 1.9% (v/v) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, when the flakes became invisible to the solution, it was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00048] [Sulfuric Acid + NaTPP] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the

flakes with water, sulfuric acid between 0.3% and 1.9% (v/v) was added. When the flakes became invisible to the solution, a solution of sodium tripolyphosphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00049] [Sulfuric Acid + Ammonium Sulphate] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, sulfuric acid between 0.3% and 1.9% (v/v) was added. When the flakes became invisible to the solution, a solution of ammonium sulphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00050] [Nitric Acid + Acetic Acid + Hydrochloric Acid + Sulfuric Acid] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid, nitric acid, hydrochloric acid, sulfuric acid having a ratio of 1:1:0:0, 1:0:1:0, 1:0:0:1, 1:1:1:0, 1:1:2:0, 1:1:1:1, 1:1:1:2 and a total v/v acid strength between 0.3% and 1.9% was added. The temperature and agitation was maintained constantly throughout the reaction for up to 4 hours. At the end, when the flakes became invisible to the solution, it was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cool down to 77 degree F, and a solution of potassium sorbate was added (1% v/v) and mixed well using agitation.

[00051] [Nitric Acid + Acetic Acid + Hydrochloric Acid + Sulfuric Acid + NaTPP] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid, nitric acid, hydrochloric acid, sulfuric acid having a ratio of 1:1:0:0, 1:0:1:0, 1:0:0:1, 1:1:1:0, 1:1:2:0, 1:1:1:1, 1:1:1:2 and a total v/v acid strength between 0.3% and 1.9% was added. When the flakes became invisible to the solution, a solution of sodium tripolyphosphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

[00052] [Nitric Acid + Acetic Acid + Hydrochloric Acid + Sulfuric Acid + Ammonium Sulphate] A germ free or autoclaved water was charged to the tank having agitation speed of 400 rpm. When the temperature reaches between 68 and 194 degrees F, chitosan flakes (0.1 - 5% w/v) having 80% DDA but not less than 75% DDA were added. The source of chitosan can be fungus, shrimp, crab or lobster or a mixture of them. Furthermore, the chitosan variants can be alpha, beta or gamma or a mixture of them. Following the soaking of the flakes with water, acetic acid, nitric acid, hydrochloric acid, sulfuric acid having a ratio of 1:1:0:0, 1:0:1:0, 1:0:0:1, 1:1:1:0, 1:1:2:0, 1:1:1:1, 1:1:1:2 and a total v/v acid strength between 0.3% and 1.9% was added. When the flakes became invisible to the solution, a solution of ammonium sulphate (0.001 - 1% v/v or v/w) was added. The temperature and agitation were maintained constantly throughout the reaction for up to 4 hours. At the end, the solution was filtered with 200 mesh followed by <635 mesh filtration unit. The solution temperature was cooled down to 77 degree F, and a solution of potassium sorbate was added (0.01 - 1% v/v) and mixed well using agitation.

EXAMPLE 2

[00053] Various of the above chitosan solutions were prepared using chitosan from two different sources. These sources are designated Tg and CTg. Tg and CTg were used to create chitosan-based nanoparticles using the above identified procedures to yield the following products

TgA = Tg source chitosan-based nanoparticles prepared with acetic acid.

TgB = Tg source chitosan-based nanoparticles prepared with acetic acid and sodium tripolyphosphate.

TgC = Tg source chitosan-based nanoparticles prepared with acetic acid and nitric acid.

TgD = Tg source chitosan-based nanoparticles prepared with acetic acid, nitric acid, and sodium tripolyphosphate.

TgE = Tg source chitosan-based nanoparticles prepared with nitric acid and sodium tripolyphosphate.

CTgA = CTg source chitosan-based nanoparticles prepared with acetic acid.

CTgB = CTg source chitosan-based nanoparticles prepared with acetic acid and sodium tripolyphosphate.

CTgC = CTg source chitosan-based nanoparticles prepared with acetic acid and nitric acid.

CTgD = CTg source chitosan-based nanoparticles prepared with acetic acid, nitric acid, and sodium tripolyphosphate.

CTgE = CTg source chitosan-based nanoparticles prepared with nitric acid and sodium tripolyphosphate.

[00054] The formulation and intermediates were characterized for the physicochemical characterizations. The physical morphology (shape and size) was characterized using the transmission or scanning electron microscope (Figures 1 and 2). Resultant solution/s obtained for the Tg sourced chitosan had > 95% particles were in the spherical, nearly spherical shape or small linear fragments having size between 0.1 nm and 5 microns. More specifically, the desired size obtained and utilized for further formulation development was less than 0.5 micron was used for further formulation development and/or other agricultural use for crop growth, stimulation, germination, and trait improvement purpose. Similarly, resultant solution obtained for the CTg sourced chitosan had the physical structure as spherical shape and size ranges between 0.1 nm and 8 microns. More specifically the size obtained between 0.1 nm and 200 nm was used as a selection parameter for the delivery to the crop for growth, stimulation, germination, and trait improvement purposes.

The dynamic stability and hydrodynamic size in the solution was characterized using the dynamic light scattering or DLS the nanoparticles were subjected to dynamic light scattering (DLS) and analyzed for Z average, Intensity, Number, and Polydispersity (PDI) (Figure 3). Resultant solutions had ranges between 0.1 nm and 10 microns with polydispersity index between 0.01 to 0.99. Furthermore, the surface zeta potential of the resultant solutions characterized by DLS (Figure 4) and it ranged between +5 and 70 mV. The major determinant factor for zeta potential was chitosan concentration and pH of the solution. Higher chitosan concentration and lower pH yielded higher zeta potential.

EXAMPLE 3

The ability of the various chitosan-based nanoparticles generated in Examples 1 and 2 to load nitrogen and/or nitrogen rich compound and/or mixture of compounds such as but not limited to potassium nitrate, calcium nitrate, magnesium nitrate, urea nitrate, urea, urea ammonia nitrate, ammonium nitrate, ammonium sulfate, Urea-hydroxyapatite, amino acids such as arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, valine, glutamine, glutamic acid, glycine, proline, taurine, aspartic acid, serine and alanine was tested. Encapsulation and slow release of nutrients is one method for balancing the nutritional needs of plants. Several solutions were prepared and tested.

Preparation of chitosan solutions

[00055] Preparation for solution A: Chitosan solution in Acetic Acid

[00056] Step 1. Take 150 ml water in a 250 mL beaker. Step 2. Turn on Top head stirrer at room temperature at 300 RPM. The stirrer was continuously ON through the process until it says stirrer STOP. Step 3. After 3 minutes of step 2, Add 8 gram chitosan flake. Step 4. After 7 minutes of step 3, Add 8 mL of 99.7 % Glacial Acetic Acid. Step 5. After complete dissolution of the flake (after 17 minutes after step 4), add 50 mL water. Step 6. After 10 minutes of step 5, The stirrer stopped. Step 7. Filter the solution with 200 mesh size filter. The total volume of the solution was 210 mL and 104.335 g/100 mL. The calculated % of the Chitosan of the solution is 3.8% (w/v). The pH of the solution is 3.88.

[00057] Preparation for solution B: Chitosan solution in HCL

[00058] Step 1. Take 150 ml water in 250 mL beaker. Step 2. Turn on Top head stirrer at room temperature at 300 RPM. The stirrer was continuously ON through the process until it says stirrer STOP. Step 3 After 3 minutes of step 2, Add 8 gram chitosan flake. Step 4. After 7 minutes of the step 3, Add 8 mL of 35.4 % HCL. Step 5. After complete dissolution of the flake (after 85 minutes after step 4), added 50 mL water. Step 6. After 26 minutes of the step 5, The stirrer stopped. Step 7. Filter the solution with 200 mesh size filter. The total volume of the solution in mL was 222.096 mL at 105.37 Gram/100 mL. The calculated % CS of the solution was 3.6 % (w/v) with a pH of the solution at 0.66.

[00059] **Preparation for solution C: Chitosan solution in Nitric Acid**

[00060] Step 1. Take 800 ml water in 2000 mL beaker. Step 2. Turn on Top head stirrer at room temperature at 600 RPM. The stirrer was continuously ON through the process until it says stirrer STOP. Step 3. After 2 minutes of step 2, Add 20 gram chitosan flake. Step 4. After 5 minutes of the step 3, Add 20 mL of 69.71 % Nitric Acid. Step 5. After complete dissolution of the flake (after 110 minutes after step 4), added 100 mL water. Step 6. After 14 minutes of the step 5, The stirrer stopped. Step 7. After 10 minutes of step 6, add 80 ml of water to make up the solution to 1000 mL. Step 8. Filter the solution with 200 mesh size filter. The total volume of the solution in mL was 1000 mL at 100.686 Gram/100 mL. The calculated % CS of the solution is 2 % (w/v) with a pH of the solution at 0.84.

[00061] **Preparation for solution D: Chitosan solution in Acetic Acid**

[00062] Step 1. Take 800 ml water in 2000 mL beaker. Step 2. Turn on Top head stirrer at room temperature at 600 RPM. The stirrer was continuously ON through the process until it says stirrer STOP. Step 3. After 4 minutes of step 2, Add 20 gram chitosan flake. Step 4. After 10 minutes of the step 3, Add 20 mL of 99.7 % Glacial Acetic Acid. Step 5. After complete dissolution of the flake (after 10 minutes after step 4). Step 6. After 17 minutes of the step 5, The stirrer was stopped. Step 7. After 10 minutes of the step 6, Add water 180 ml to make up the solution to 1000 mL. Step 8. Filter the solution with 200 mesh size filter. The total volume of the solution in mL was 1000 ML at 100.472 Gram/100 mL. The calculated % CS of the solution was 2 % (w/v) with a pH of the solution at 3.91.

[00063] **Preparation for solution E: Chitosan solution in H₂SO₄**

[00064] The H₂SO₄ solution did not work, as the chitosan did not dissolve in solution, this effectively terminated the experiment, and the H₂SO₄ solution was not used in any encapsulation production.

[00065] **Preparation for solution F: Chitosan solution in HCL**

[00066] Step 1. Take 800 ml water in 2000 mL beaker. Step 2. Turn on Top head stirrer at room temperature at 600 RPM. The stirrer was continuously ON through the process until it says stirrer STOP. Step 3. After 2 minutes of step 2, Add 20 gram chitosan flake. Step 4. After 6 minutes of the step 3, Add 20 mL of 35.4 % HCL. Step 5. After complete dissolution of the flake (after 44 minutes after step 4), added 100 mL water. Step 6. After 12 minutes of the step 5, The stirrer stopped. Step 7. After 10 minutes of the step 6, Added 80 mL water to make up to 1000 mL and mixed thoroughly. Step 8. Filter the solution with 200 mesh size filter. The total volume of the solution in mL was 1000 mL at 100.328 Gram/100 mL. The calculated % CS of the solution was 2% (w/v) with a pH of the solution at 1.09.

[00067] **Preparation of Linker - Ammonium sulphate (AS) solution**

[00068] Step 1. Take 0.200 gram ammonium sulphate in 50 mL capacity volumetric flask. Step 2. Add 30 mL water. Step 3. Vortex the solution – resultant solution becomes clear. Step 4. Make up the volume to 50 mL using 20 mL water. The total volume of the solution in mL was 50 mL; 49.764 g. The calculated % AS of the solution was 0.4 % w/v. The calculated N % of the solution was 0.0856% w/v. The calculated the S % of the solution was 0.0980% w/v. The pH of the solution was 5.75.

[00069] **Preparation of Linker - Tri sodium Polyphosphate (TPP) solution**

[00070] Step 1. Take 0.200 gram in 50 mL capacity volumetric flask. Step 2. Add 30 mL water. Step 3. Vortex the solution – resultant solution becomes clear. Step 4. Make up the volume to 50 mL using 20 mL water. The total volume of the solution in mL was 50 mL; 49.821 g. The calculated % TP of the solution was 0.4 % w/v. The calculated P% of the solution was 0.101%. The pH of the solution was 9.76.

[00071] Formulation of the Encapsulated Urea

[00072] **Align N # 1**

[00073] Step 1. Take 2000 mL beaker. Step 2. Add 175 mL solution A. Step 3. Start over head stirrer, it was continued through the process until it says stop. Step 4. After 5 minutes of the step 3, add 100 mL water. Step 5. After 6 minutes of the step 4, add 100 gram urea. Step 6. After 13 minutes of the step 5, add 100 gram urea. Step 7. After 1 minutes of the step 6, add 50 mL water. Step 8. After 8 minutes of the step 7, add 50 mL water. Step 9. After 10 minutes of the step 8, add 135 gram urea. Step 10. After 9 minutes of the step 9, add 175 solution B. Step 11. After 18 minutes of step 10, add 25 mL water. Step 12. After 52 minutes of the step 11, add 10 mL Ammonium sulphate solution (0.1 gram in 10 mL or 1% w/v). Step 12. After 15 minutes of the step 12, stirrer was stopped. The solution makes up to 1000 mL using 125 mL water. Step 13. Filter the solution with 200 mesh size filter. Step 14. Solution is ready for further use/ QC testing analyses, stored in 1 L bottle. The method resulted in chitosan encapsulation of the nutrients, with the solution having Urea: 43.5 % (w/v), N % w/v: 20.01 (calculated); 18.24% (analysed by TKN), Ammonium sulphate: 0.01 (w/v), Chitosan: 1.29 % (w/v), Acetic Acid: 0.7 % (v/v), HCl: 0.7 % (v/v), pH: 3.67. Weight (gram/100 mL): 110.832 gram

[00074] **Align N # 2**

[00075] Step 1. Take 2000 mL beaker. Step 2. Add 200 mL solution D. Step 3. Started over head stirrer, it was continued through the process until it says stop. Step 4. After 3 minutes of the step 3, add 200 mL distil water. Step 5. After 5 minutes of the step 4, add 100 gram urea. Step 6. After 7 minutes of the step 5, add 100 gram urea. Step 7. After 5 minutes of the step 6, add 100gram urea. Step 8. After 8 minutes of the step 7, add 135 gram urea. Step 9. After 10 minutes of the step 8, add 100 solution F. Step 10. After 13 minutes of the step 9, add 100 solution F. Step 11. After 19 minutes of step 10, add 75 mL water. Step 12. After 5 minutes of the step 11, add 25 mL Ammonium sulphate solution (0.1 gram in 25 mL water or 0.4% w/v). Step 13. After 15 minutes of the step 12, stirrer was stopped. The solution was 1 L. Step 14. Filter the solution with 200 mesh size filter. Step 15. Solution is ready for further use/ QC testing analyses, stored in 1 L bottle. This example of the method resulted in chitosan encapsulation of the nutrients, with the solution having, Urea: 43.5 % (w/v), N % w/v: 20.01 (calculated); 18.05 (analysed by TKN), Ammonium sulphate: 0.01 (w/v), Chitosan: 0.8 % (w/v), Acetic Acid: 0.4 % (v/v), HCl Acid: 0.4 % (v/v), pH: 4, and a Weight (gram/100 mL): 110.622 gram.

[00076] **Align N # 3**

[00077] **Step 1.** Take 2000 mL beaker. Step 2. Add 200 mL water. Step 3. Started over head stirrer, it was continued through the process until it says stop. Step 4. After 2 minutes of the step 3, add 200 mL solution D. Step 5. After 5 minutes of the step 4, add 100 gram urea. Step 6. After 3 minutes of the step 5, add 100 gram urea. Step 7. After 6 minutes of the step 6, add 100 gram urea. Step 8. After 11 minutes of the step 7, add 135 gram urea. Step 9. After 10 minutes of the step 8, add 200 solution C. Step 10. After 50 minutes of the step 9, add Ammonium sulphate solution (0.1 gram in 25 mL water or 0.4% w/v). Step 11. After 35 minutes of the step 10, stirrer was stopped. The solution was made up to 1000 mL, by adding 60 mL water. Step 12. Filter the solution with 200 mesh size filter. Step 13. Solution is ready for further use/ QC testing analyses, stored in 1 L bottle. This example of the method resulted in chitosan encapsulation of the nutrients, with the solution having; Urea: 43.5 % (w/v), N % w/v: 20.1 (calculated); 18.17 (analysed by TKN), Ammonium sulphate: 0.01% (w/v), Chitosan: 0.8 % (w/v), Acetic Acid: 0.4 (v/v), Nitric Acid: 0.4 (v/v), a pH:3.22, and a Weight (gram/100 mL): 110.622 gram.

[00078] **Align N # 4**

[00079] Step 1. Take 1000 mL beaker. Step 2 Add 300 mL water. Step 3. Start magnetic stirrer, it was continued through the process until it says stop. Step 4. After 07 minutes of the step 3, add 100 gram urea. Step 5. After 4 minutes of the step 4, add 100 gram urea. Step 6. After 2 minutes of the step 5, add 100 gram urea. Step 7. After 4 minutes of the step 6, add 135 gram urea. Step 8. After 15 minutes of the step 7 (solution was not completely dissolved like semi crystals appearing bottom of the beaker), and stirrer stop. Step 9. After step 8, Take another separate 2 L beaker and added 200 mL solution C and started overhead stirrer. Step 10. After 2 minutes of step 9, add semi dissolve solution of urea (prepared in the step 7) transfer into solution C. Step 11. After 3 minutes of step 10, add 100 mL water. Step 12. After 17 minutes of step 11, add 50 mL water. Step 13. After 8 minutes of the step 12, added ammonium sulphate solution (0.1 gram in 25 mL water or 0.4% w/v). Step 14. After 25 minutes of the step 13, stirrer was stopped. The solution was make up to 1000 mL for that 25 mL water was added. Step 15. Filter the solution with 200 mesh size filter. Step 16. Solution is ready for further use/ QC testing analyses, stored in 1 L bottle. This example of the method resulted in chitosan encapsulation of the nutrients, with the solution having; Urea: 43.5 % (w/v), N % w/v: 20.01 (calculated); 18.42 (analysed by TKN), Ammonium sulphate: 0.01 (w/v), Chitosan: 0.4 % (w/v) Nitric Acid: 0.4% (v/v), pH: 2.87, and Weight (gram/100 mL): 110.586 gram.

[00080] **Align N # 5**

[00081] Step 1. Take 2000 mL beaker. Step 2. Add 200 mL solution D. Step 3. Started over head stirrer, it was continued through the process until it says stop. Step 4. After 2 minutes of the step 3, add 200 mL water. Step 5. After 4 minutes of the step 4, add 100 gram urea. Step 6. After 4 minutes of the step 5, add 100 gram urea. Step 7. After 12 minutes of the step 6, add 100gram urea. Step 8. After 11 minutes of the step 7, add 135 gram urea. Step 9. After 13 minutes of the step 8, add 200 solution C. Step 10. After 25 minutes of the step 9, add TPP solution (0.1 gram TPP in 25 mL water or 0.4% w/v). Step 11. After 20 minutes of the step 10, stirrer was stopped. The solution was made up to 1000 mL by adding 80 mL water. Step 12. Filter the solution with 200 mesh size filter. This example of the method resulted in chitosan encapsulation of the nutrients, with the solution having; Urea: 43.5 % (w/v), N % w/v: 20.01 (calculated); 18.06 (analysed by TKN), TPP: 0.01% (w/v), Chitosan: 0.8 % (w/v), Acetic Acid: 0.4 % (v/v), Nitric Acid: 0.4 % (v/v), pH: 3.14, and a Weight (gram/100 mL): 110.726 gram.

[00082] **Align N # 6**

[00083] Step 1. Take 1000 mL beaker. Step 2. Add 400 mL water. Step 3. Started magnetic (bottom head) stirrer, it was continued through the process until it says stop. Step 4. After 5 minutes of the step 3, add 100 gram urea. Step 5. After 9 minutes of the step 4, add 100 gram urea. Step 6. After 4 minutes of the step 5, add 100 gram urea. Step 7. After 12 minutes of the step 6, add 135 gram urea. Step 8. After 30 minutes of the step 7 (solution was not completely dissolved, crystals were appearing at bottom of the beaker), and stirrer stop. Step 9. Take another separate 2 L volume beaker and added 200 mL solution C. The overhead stirrer was started. Step 10. After 4 minutes of step 9, added urea semi dissolve solution (prepared in step 7) transfer into solution C. Step 11. Step 12. After 1 minutes of step 10, add 50 mL water. Step 13. After 15 minutes of the step 11, add TPP solution (0.1 gram in 25 mL water or 0.4% w/v). Step 14. After 20 minutes of the step 12, stirrer was stopped. Step 15. The solution was made up to 1000 mL by adding 25 mL water. Step 16. Filter the solution with 200 mesh size filter. This example of the method resulted in chitosan encapsulation of the nutrients, with the solution having; Urea: 43.5 % (w/v), N % w/v: 20.01 (calculated); 17.9 (analysed by TKN), TPP: 0.01 % (w/v), Chitosan: 0.4% (w/v), Nitric Acid: 0.4% (v/v), pH 2.88, Weight (gram/100 mL): 110.506 gram.

[00084] **Align N # 7**

[00085] Step 1. Take 1000 mL beaker. Step 2. Add 400 mL water. Step 3. Start magnetic stirrer, it was continued through the process until it says stop. Step 4. After 5 minutes of the step 3, add 100 gram urea. Step 5. After 10 minutes of the step 4, add 100 gram urea. Step 6. After 20 minutes of the step 5, add 100 gram urea. Step 7. After 10 minutes of the step 6, add 135 gram urea. Step 8. After 305 minutes of the step 7 (solution was not completely dissolved like semi crystals appearing bottom of the beaker), and stirrer stop. Step 9. Take another separate 2L beaker and added 200 mL solution D, and started overhead stirrer. Step 10. After 2 minutes of step 9, add urea semi dissolve solution (prepared in the step 8) transfer into solution D. Step 11. After 3 minutes of step 10, add 25 mL water. Step 12. After 11 minutes of the step 11, add ammonium sulphate solution (0.1 gram in 25 mL water or 0.4% w/v). Step 13. After 26 minutes of the step 12, stirrer was stopped and the solution was made up to 1000 mL by adding 50 mL water. Step 14. Filter the solution with 200 mesh size filter. This example of the method resulted in chitosan encapsulation of the nutrients, with the solution having; Urea: 43.5 % (w/v), N % w/v : 20.01 (calculated); 18.19 (analysed by TKN), Ammonium sulphate: 0.01 % (w/v), Chitosan: 0.4 % (w/v), Acetic Acid: 0.4 % (v/v), pH : 4.76, and Weight (gram/100 mL): 110.29 gram.

[00086] pH analysis (Figure 5) was performed according to standard protocols for drinking water as described by the United States EPA. Total Kjendahl Nitrogen (TKN) analysis (Figure 5) was performed in accordance with method 1688 of the U. S. EPA Office of Water. The UV-Vis analysis (Figure 6) was performed according to OPPTS 830.7050. The FTIR analysis (Figures 7-13) was performed on and according to the manual for the Agilent-Cary 630.

Example 4: Application and uses of the chitosan and/or chitosan/nitrogen nanoparticles The resultant chitosan and/or chitosan loaded nitrogen nanoparticle products or mixtures of products obtained as a result of the above Examples were used for the multidimensional effects on plants such as growth, protection, and nutrition. The multidimensional effects on plants were obtained by applying the resultant product of the above Examples onto the seed surface, mixing in the soil, exposing root and/or exposing leaves. The application rate ranged between 0.01% to 50% v/w or v/v. Furthermore, the resultant products of the above Examples were used to mix with other agrochemicals to act as growth promoter, symbiont, nutrient, protection agent against pathogens or non-desired plants for synergistic effects.

[00087] The invention has been described with reference to various specific and preferred embodiments and techniques. Nevertheless, it is understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

WHAT IS CLAIMED IS:

1. A composition comprising: chitosan and at least 1% (w/v) nitrogen.
2. The composition of claim 1, wherein at least 50% of the nitrogen is in the chemical form of amine and/or nitrate and/or ammonia.
3. The composition of claim 2, wherein the composition comprises nanoparticle of chitosan and a nitrogen rich compound and/or mixture of compounds selected from the group consisting of potassium nitrate, calcium nitrate, magnesium nitrate, urea nitrate, urea ammonia nitrate, ammonium nitrate, ammonium sulfate, urea-hydroxyapatite, amino acids such as arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, valine, glutamine, glutamic acid, glycine, proline, taurine, aspartic acid, serine and alanine.
4. The composition of claim 1, wherein the chitosan is present at between about 0.01% and about 1.5% (w/v).
5. The composition of claim 1, further comprising a linker.
6. The composition of claim 5, where the linker is sodium tripolyphosphate and/or ammonium sulfate.
7. A method of producing chitosan nanoparticles, the method comprising:
 - dissolving chitosan in acidic water, where the water is made acidic by an acid selected from the group of acetic acid, nitric acid, sulfuric acid, hydrochloric acid, and combinations thereof; and
 - forming nanoparticles from the dissolved chitosan.
8. The method according to claim 7, wherein a linker is added to the dissolved chitosan.
9. The method according to claim 8, wherein the linker is sodium tripolyphosphate and/or ammonium sulfate.

10. The method according to claim 7, wherein nitrogen rich compound and/or mixture of compounds selected from the group consisting of potassium nitrate, calcium nitrate, magnesium nitrate, urea nitrate, urea ammonia nitrate, ammonium nitrate, ammonium sulfate, urea-hydroxyapatite, amino acids such as arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, valine, glutamine, glutamic acid, glycine, proline, taurine, aspartic acid, serine and alanine is/are added the dissolved chitosan.

11. The method according to claim 10, wherein the nanoparticles comprise chitosan and nitrogen rich compound and/or mixture of compounds.

12. The method according to claim 11, wherein the nanoparticles comprise at least 1% (w/v) nitrogen.

13. , The method according to claim 11, wherein the chitosan is present at between about 0.01% and about 1.5% (w/v).

14. A method of treating a plant or a plant part, the method comprising treating the plant or plant part with a composition according to any of claims 1-7.

15. The method according to claim 14, wherein the plant part is selected from the group consisting of a seed, leaf, stem, flower, or root of a plant.

16. A method of preparing a plant growth medium, the method comprising treating the growth medium with a composition according to any of claims 1-7.

17. The method according to claim 16, wherein the growth medium is soil, peat, moss, wood residue, leaf mold, sawdust, bark, bagasse, rice hull, sand, perlite, vermiculite, calcinated clay, polystyrene, urea formaldehyde resins, and hydroponic liquids, and combinations thereof.

ABSTRACT

Disclosed herein are compositions comprising: chitosan and at least 1% (w/v) nitrogen. In certain cases, at least 50% of the nitrogen is in chemical form of amine and/or nitrate and/or ammonia. Also disclosed herein are methods of producing chitosan nanoparticles by dissolving chitosan in acidic water, where the water is made acidic by an acid selected from the group of acetic acid, nitric acid, sulfuric acid, hydrochloric acid, and combinations thereof; and forming nanoparticles from the dissolved chitosan. Further disclosed is the treatment of plants or plant parts with the compositions disclosed herein.

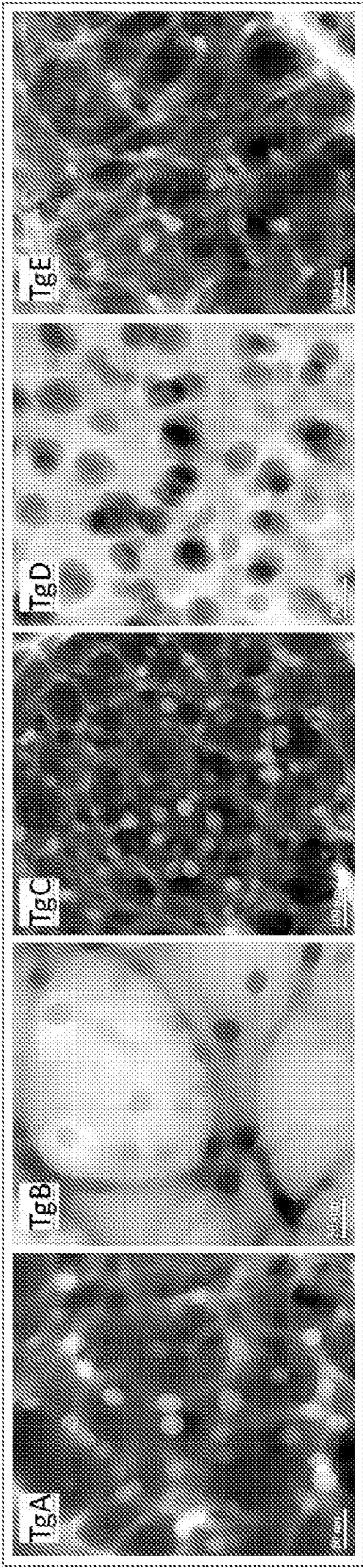


Fig. 1

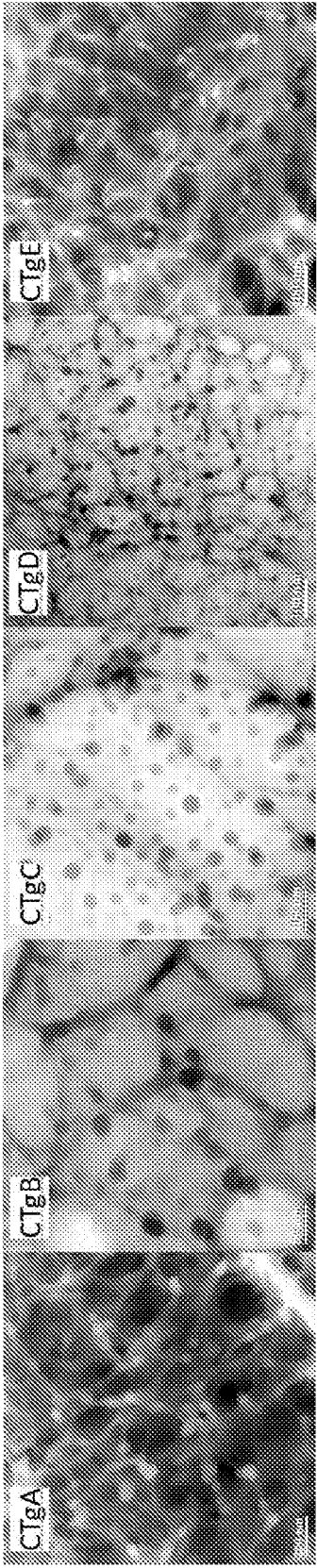


Fig. 2

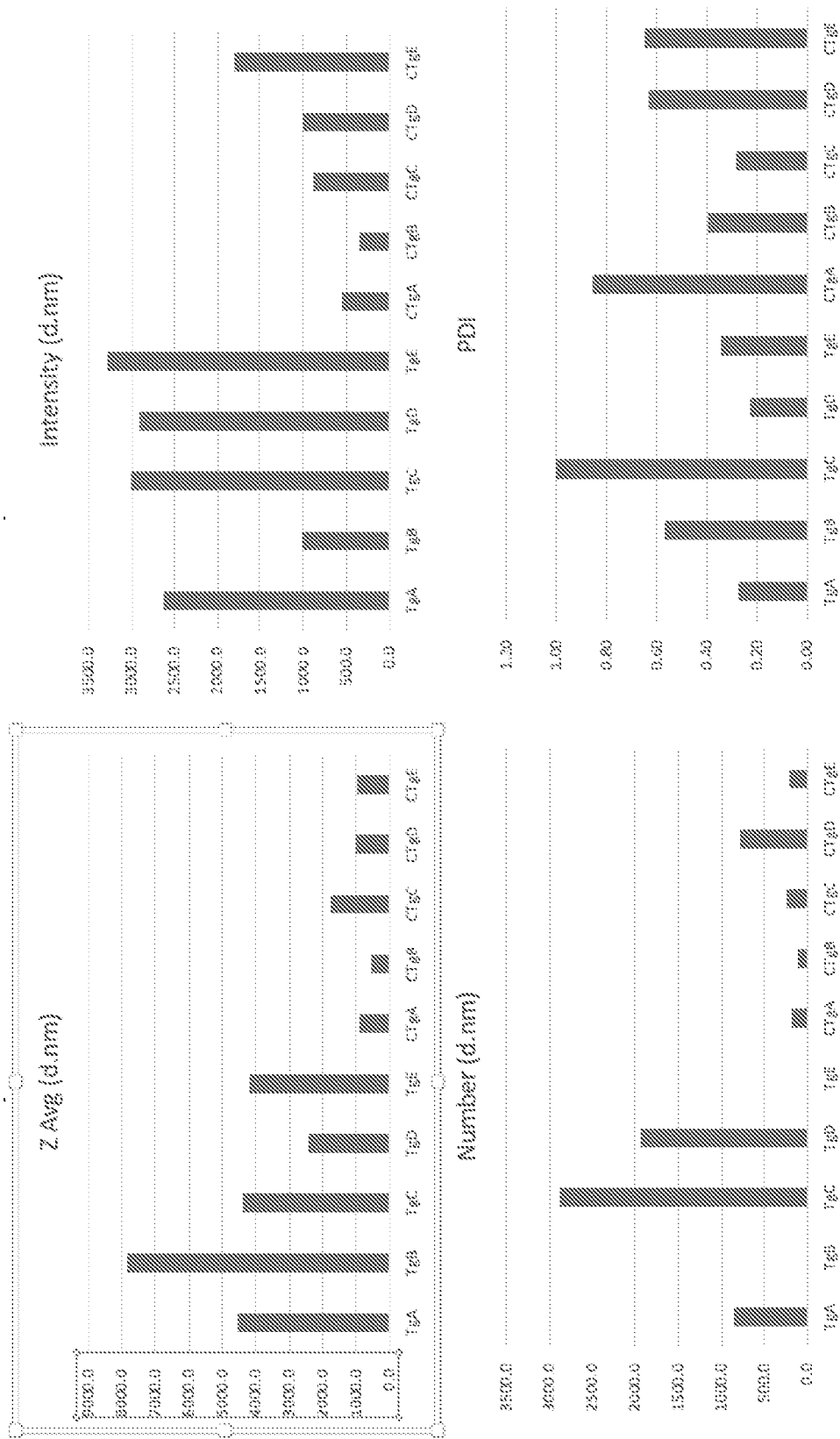


Fig. 3

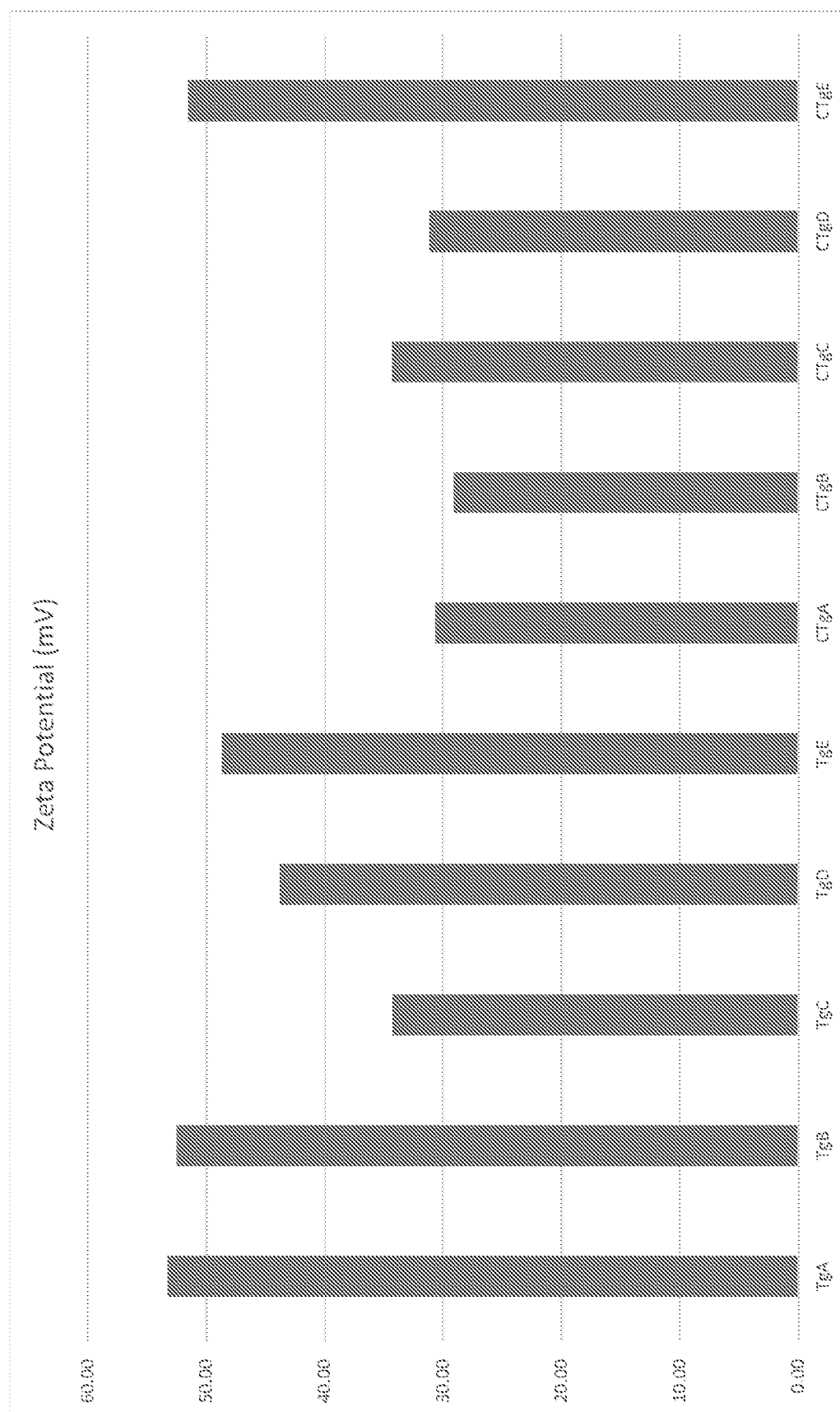


Fig. 4

Align Batch #	pH	TKN% (w/v)
1	3.67	18.24
2	4	18.05
3	3.22	18.17
4	2.87	18.42
5	3.14	18.06
6	2.88	17.9
7	4.76	18.19
CS (soln D)	4.05	0.31
N source	8.75	18.41
TBA = To be analysed		
N source = Urea		

Fig. 5

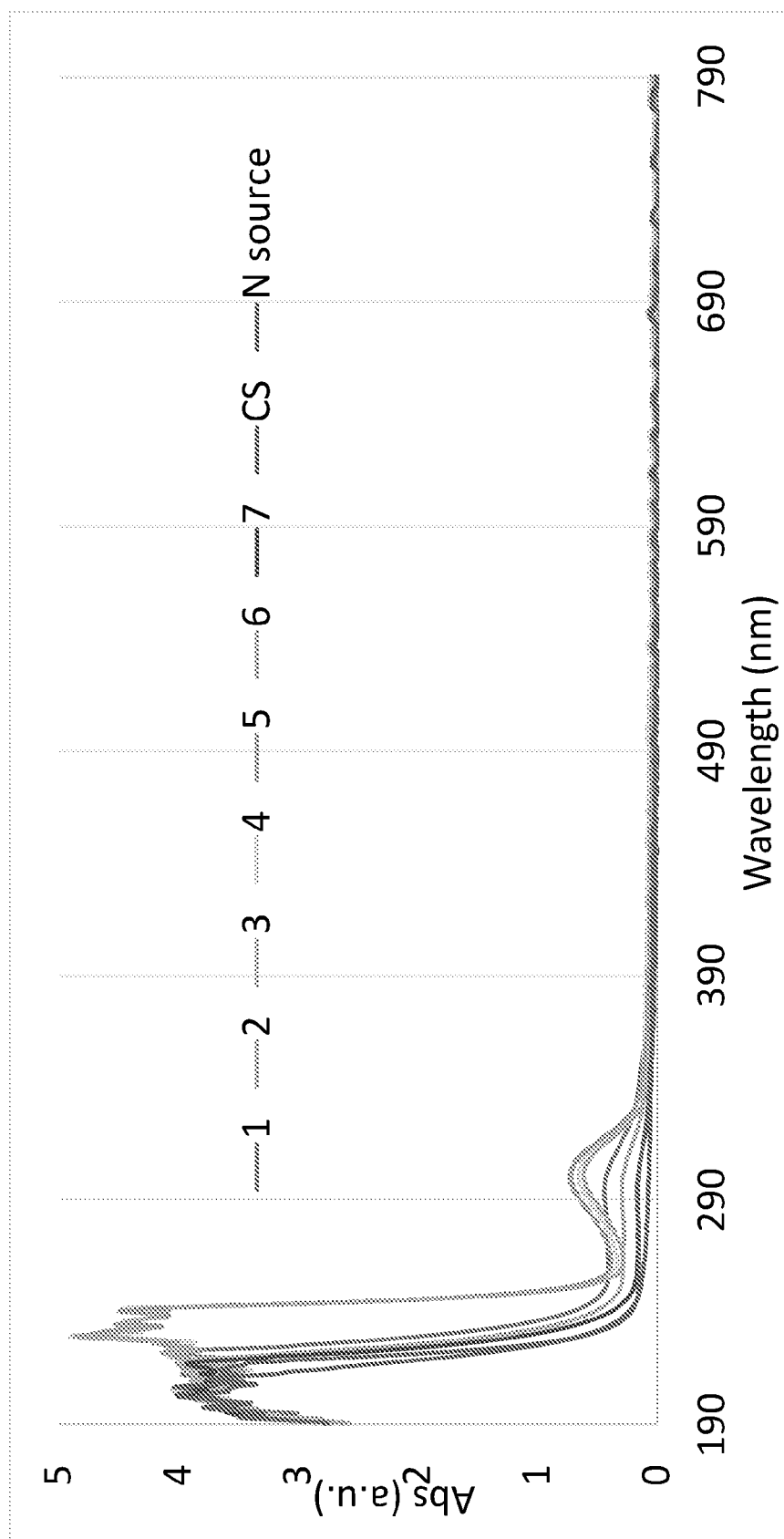


Fig. 6

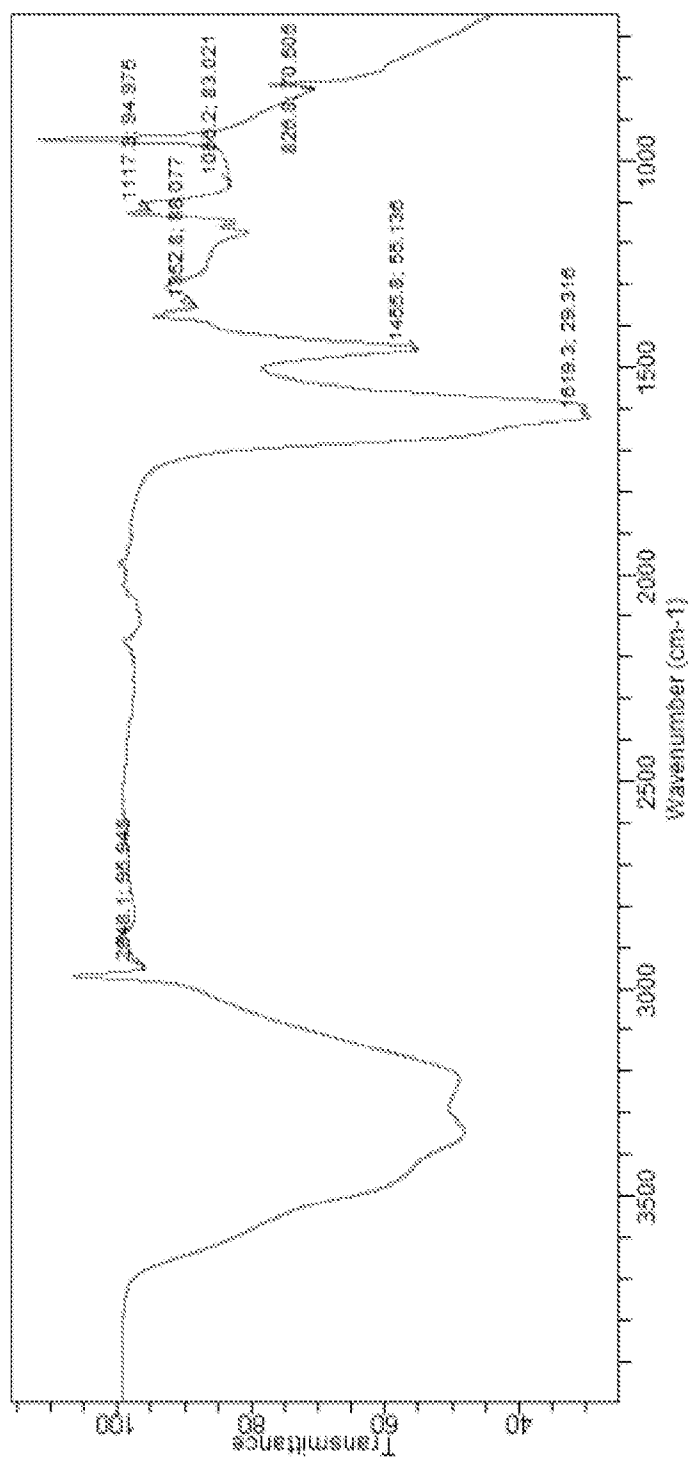


Fig. 7

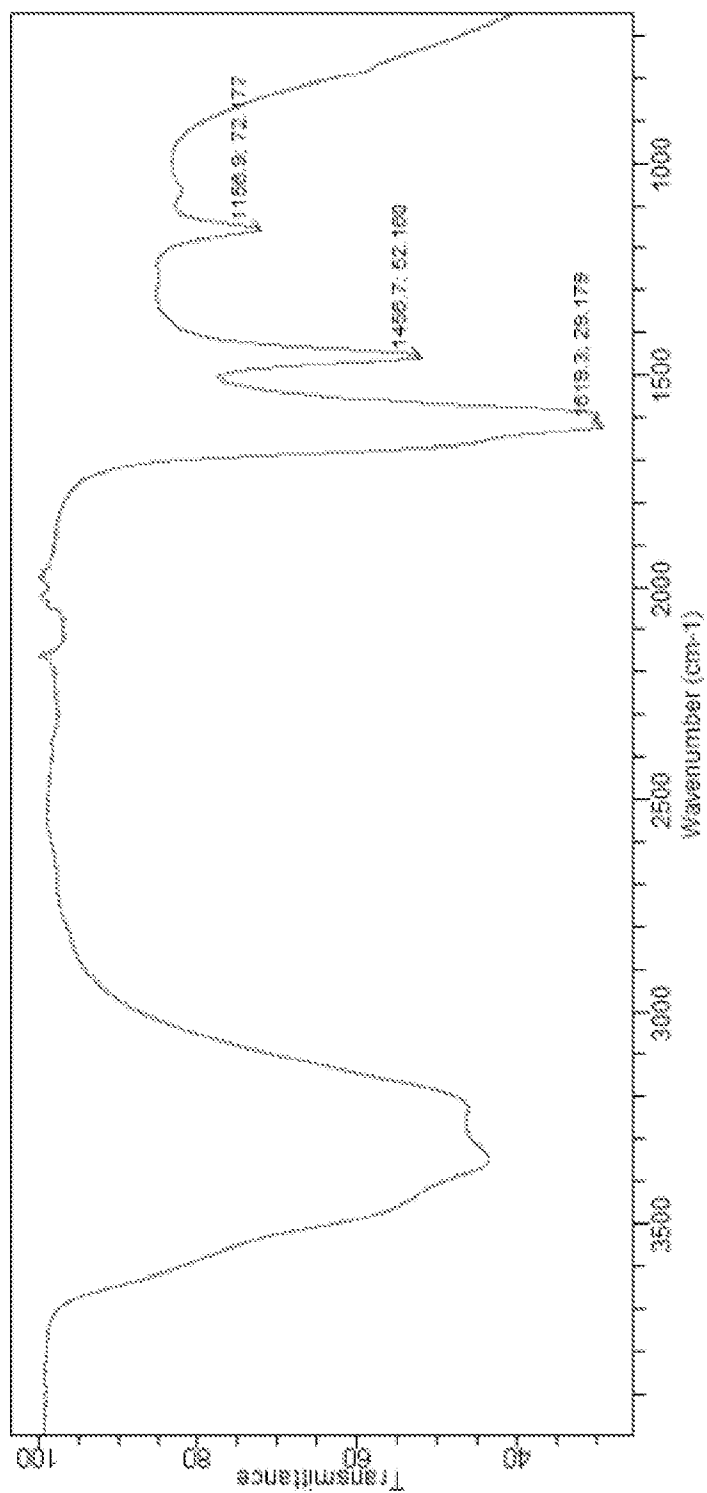


Fig. 8

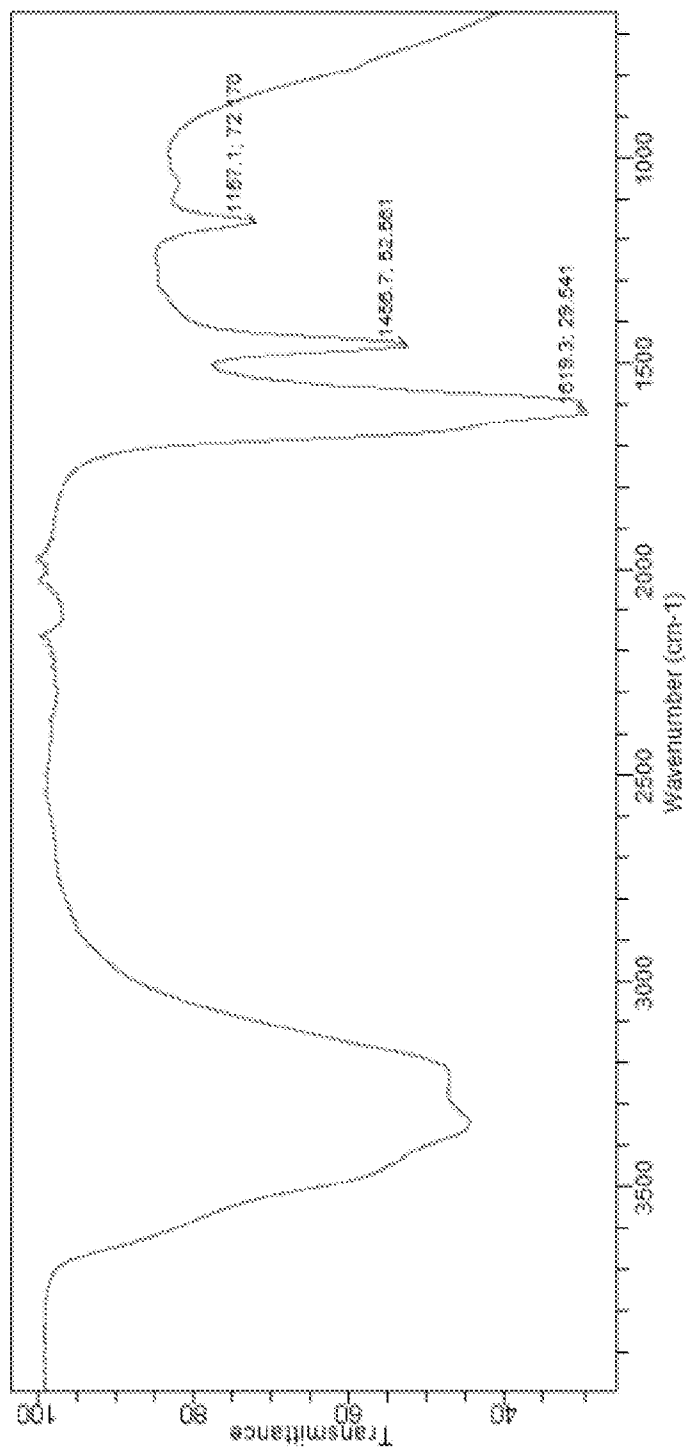


Fig. 9

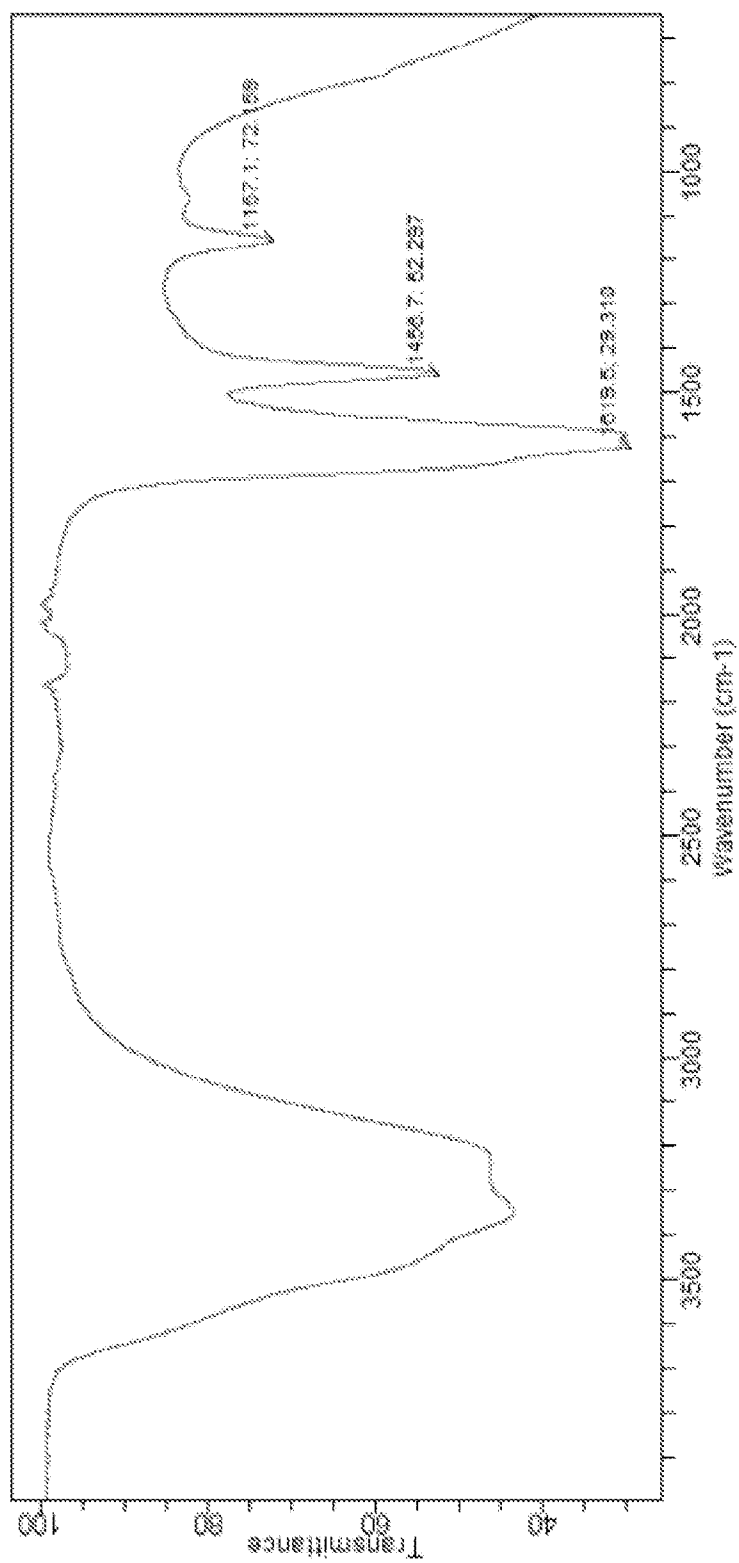


Fig. 10

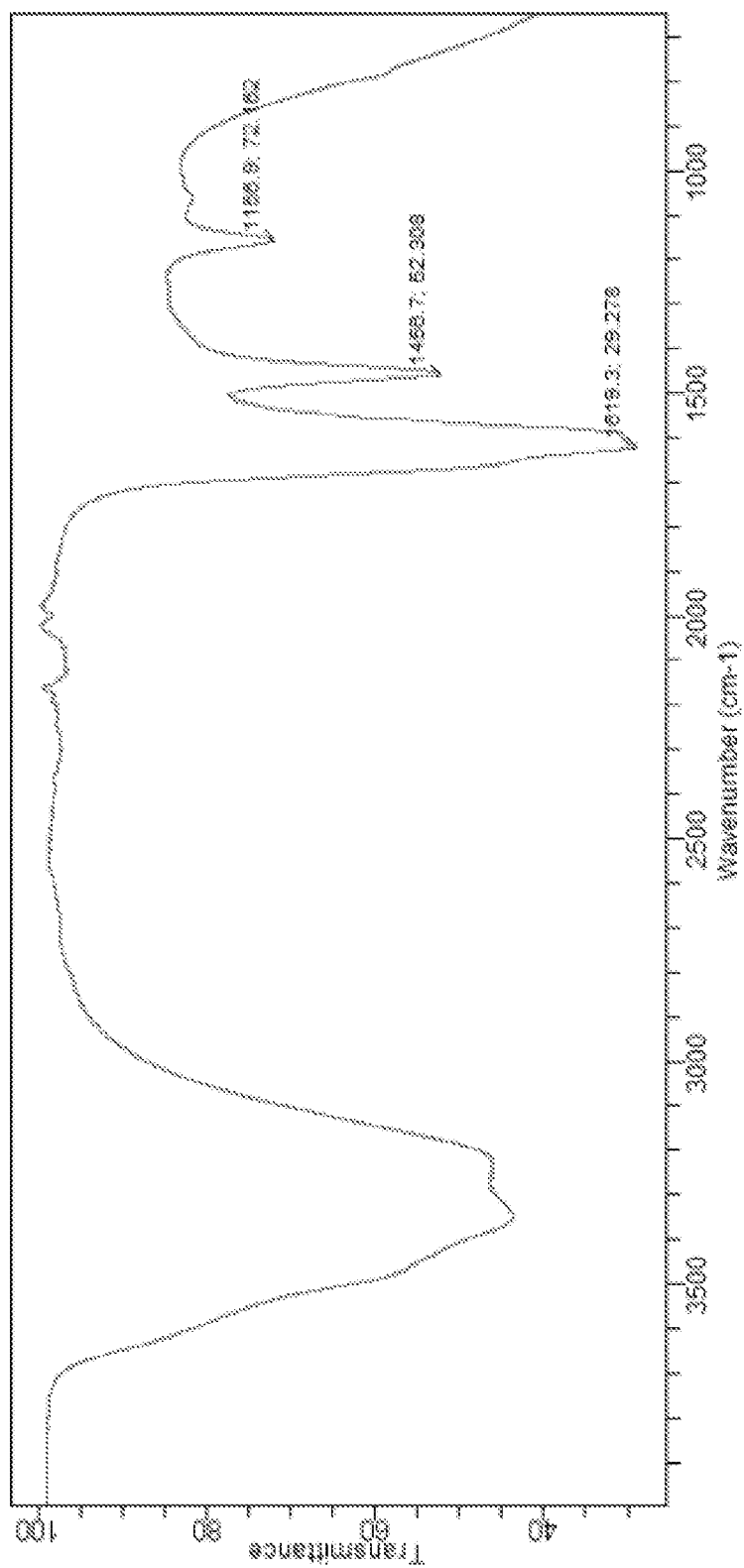


Fig. 11

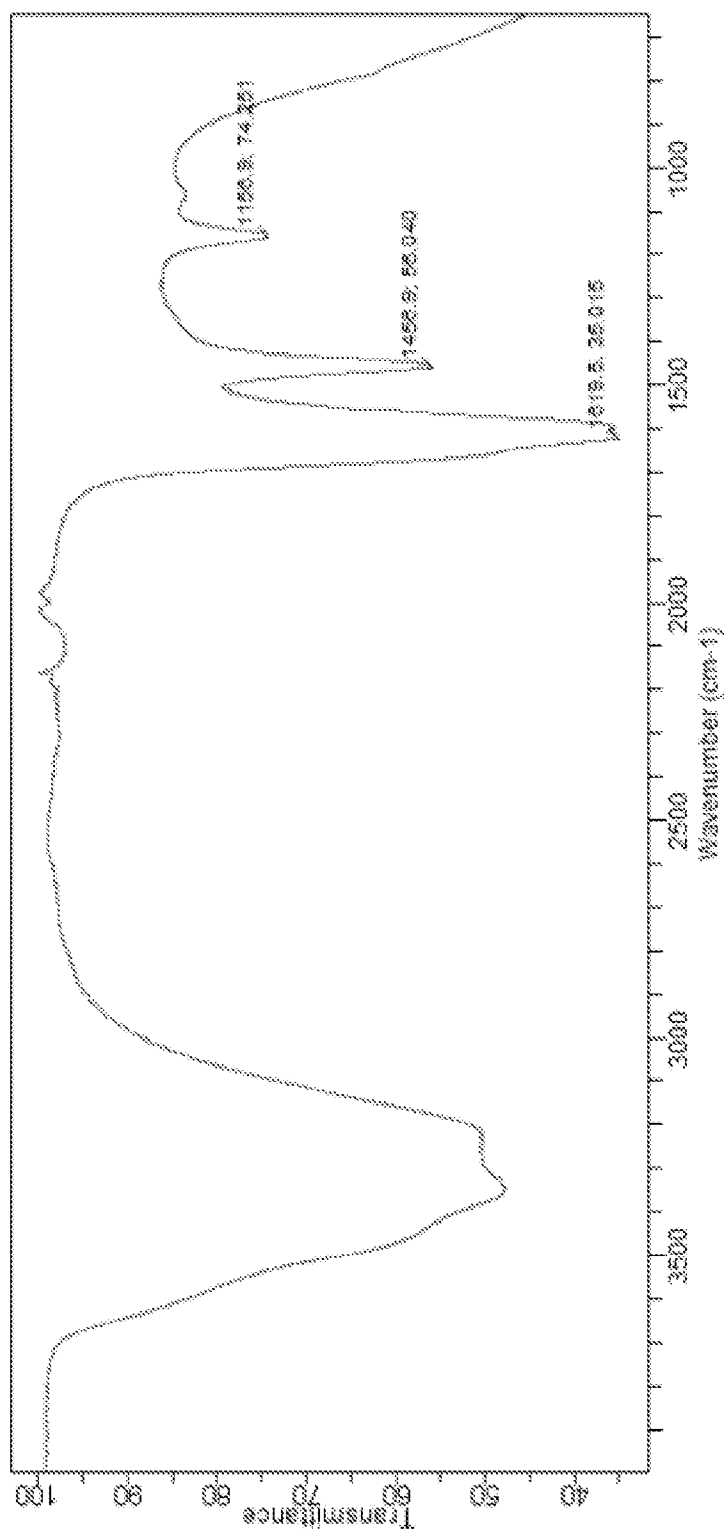


Fig. 12

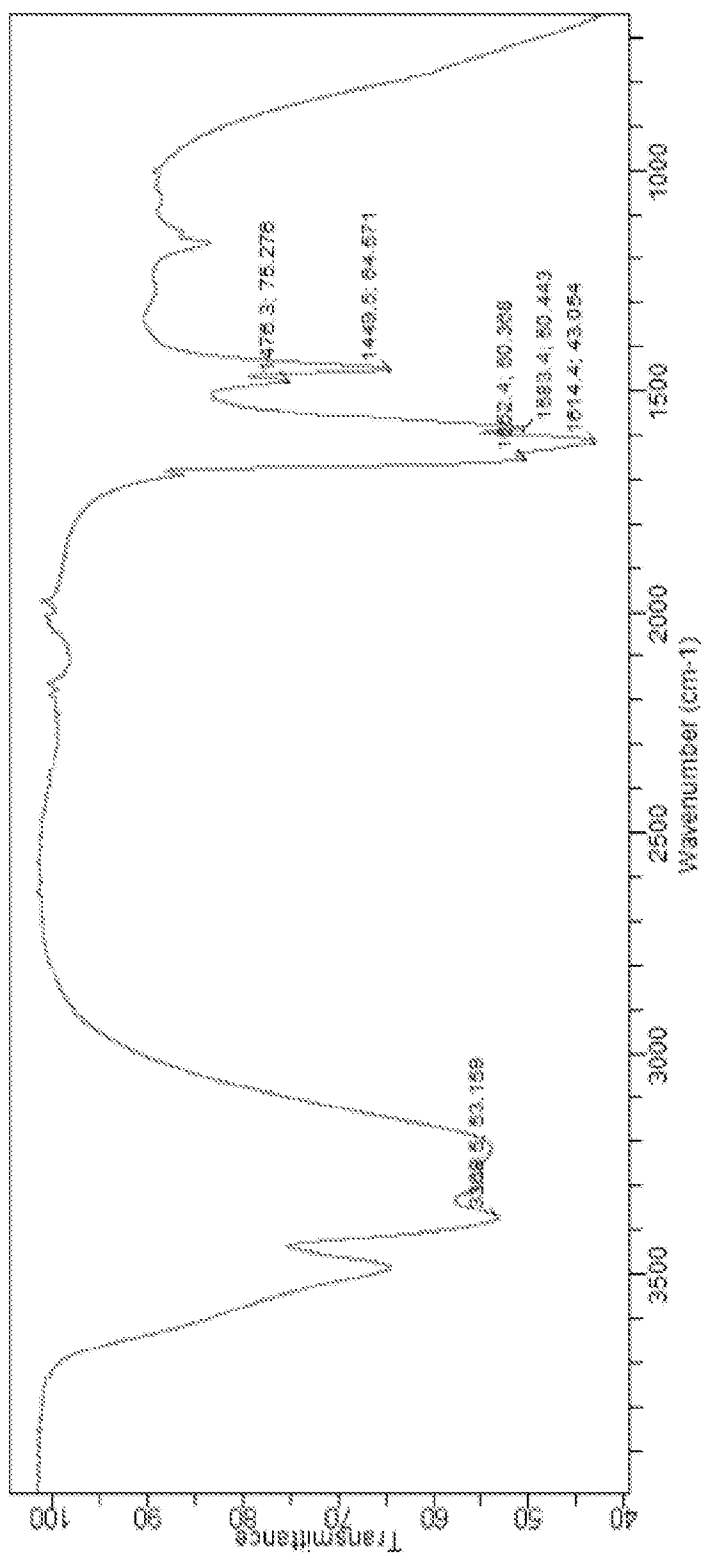


Fig. 13

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	10860-10105 PR
		Application Number	
Title of Invention	Compositions and Methods for Agri-Inputs and Uses Thereof		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2:

☐	Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)
--------------------------	---

Inventor Information:

Inventor 1 Remove				
Legal Name				
Prefix	Given Name	Middle Name	Family Name	Suffix
	Ramesh		Raliya	
Residence Information (Select One) ☐ US Residency ☒ Non US Residency ☐ Active US Military Service				
City	Ahmedabad	Country of Residence ⁱ	IN	
Mailing Address of Inventor:				
Address 1		c/o Tidal Vision Products		
Address 2		3710 Iron Gate Rd		
City	Bellingham	State/Province	WA	
Postal Code	98226	Country ⁱ	US	
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button. Add				

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).	
☐ An Address is being provided for the correspondence Information of this application.	
Customer Number	93219
Email Address	docketing@patentlawworks.net Add Email Remove Email

Application Information:

Title of the Invention	Compositions and Methods for Agri-Inputs and Uses Thereof		
Attorney Docket Number	10860-10105 PR	Small Entity Status Claimed ☒	
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	12	Suggested Figure for Publication (if any)	

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	10860-10105 PR
		Application Number	
Title of Invention	Compositions and Methods for Agri-Inputs and Uses Thereof		

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	93219		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	10860-10105 PR
		Application Number	
Title of Invention	Compositions and Methods for Agri-Inputs and Uses Thereof		

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)ⁱ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Access Code ⁱ (if applicable)

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Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	10860-10105 PR
		Application Number	
Title of Invention	Compositions and Methods for Agri-Inputs and Uses Thereof		

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	10860-10105 PR
	Application Number	
Title of Invention	Compositions and Methods for Agri-Inputs and Uses Thereof	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.			
Applicant 1			Remove
If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.			
Clear			
☒ Assignee	☐ Legal Representative under 35 U.S.C. 117	☐ Joint Inventor	
☐ Person to whom the inventor is obligated to assign.		☐ Person who shows sufficient proprietary interest	
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:			
Name of the Deceased or Legally Incapacitated Inventor:			
If the Applicant is an Organization check here. ☒			
Organization Name	Tidal Vision Products		
Mailing Address Information For Applicant:			
Address 1	3710 Iron Gate Rd		
Address 2			
City	Bellingham	State/Province	WA
Country	US	Postal Code	98226
Phone Number		Fax Number	
Email Address			
Additional Applicant Data may be generated within this form by selecting the Add button. Add			

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	10860-10105 PR
	Application Number	
Title of Invention	Compositions and Methods for Agri-Inputs and Uses Thereof	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

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Address 2				
City		State/Province		
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NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Daniel J. Morath, Ph.D./			Date (YYYY-MM-DD)	2023-05-10
First Name	Daniel J.	Last Name	Morath, Ph.D.	Registration Number	55,896

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	10860-10105 PR
		Application Number	
Title of Invention	Compositions and Methods for Agri-Inputs and Uses Thereof		

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
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8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.



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APPLICATION #
63/501,190

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ATTORNEY DOCKET #
10860-10105 PR

Title of Invention

Compositions and Methods for Agri-Inputs and Uses Thereof

Application Information

APPLICATION TYPE Utility - Provisional Application under
35 USC 111(b)

PATENT # -

CONFIRMATION # 5654

FILED BY DANIEL MORATH

PATENT CENTER # 62067610

FILING DATE -

CUSTOMER # 93219

FIRST NAMED INVENTOR Ramesh Raliya

CORRESPONDENCE ADDRESS -

AUTHORIZED BY DANIEL MORATH

Documents

TOTAL DOCUMENTS: 3

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
10098 PR 2023-05-10 - Application Data Sheet - SIGNED.pdf	8	Application Data Sheet	2174 KB
10105 PR 2023-05-10 - Specification-APP.TEXT.docx	26	Application body structured text document	54 KB
Warning: At least one claim appears to contain more than one sentence. Please review and revise if necessary. Bookmarks were found and have been removed. Text decorations have been removed.			
10105 PR 2023-05-10 - Drawings.pdf	12	Drawings-only black and white line drawings	6067 KB

Warning: The attached file contains comments or annotations. Comments are not recommended and may cause processing problems with the document.

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
10098 PR 2023-05-10 - Application Data Sheet - SIGNED.pdf	1B3125129B4A20068EF3283D4E4EC11806B5F67C303DA03267 BF55B4BCF609C2A8FF37AE6C7EBAB8CFABCE51B45101C895 62781966EAFE45F4B6D1C1656710B
10105 PR 2023-05-10 - Specification-APP.TEXT.docx	1AD797632F2382F217B2172C5688ACF07EB1DAD49BE664701 B9600DCBE3352F948D7A56A1974ED95CAAD072DF684F1461B E2C319D16B5033E51C582EA2A86BE1
10105 PR 2023-05-10 - Drawings.pdf	99CAA40418BDBEA45FDE5325D3AD6490E8F841EEDC3F160D 3C03C1AB5385A36CB9C43DD1216FE5971BD4706977E852435 9FE9B10EC2F5A44F428D22C658C2B8B

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

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If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

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