# From the Pioneers of Agricultural Chitosan Technology INTRODUCING



# Nematicide Fungicide Bactericide Biostimulant 6% Chitosan Solution

In November 2022, EPA determined that chitosan can be used in minimum risk use (also known as 25(b) pesticide formulations. Organisan Corporation is introducing its 25(b) product, Impact SIX. Organisan has leveraged its extensive experience with chitosan and chitosan formulations to create a novel product with multiple activities. Impact SIX features 6% chitosan. Impact SIX boasts four major claims, nematicide, fungicide, bacteriocide and biostimulant. Such a wide spectrum of actions is possible due to the specialized formulation of Impact SIX.



# The Bridge to Regenerative Agriculture

Delivers multiple modes of chitosan technology and efficacy to you in one uniquely-tailored product.

# **Enhances Natural Defenses**

Helps your plants' ability to fight both biotic and abiotic stress such as drought excess moisture and others.

## **SAR Response**

Stimulates phytoalexin responses.

## **Provides Overall Improvement**

Contributes to the overall enhancement of plant health, productivity and stamina.

## **Produces Greater Outcomes**

Works in harmony with nature to produce higher yields and opens previously inaccessible pathways to nutrients in your soil.





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# IMPACT

as a Bridge To Regenerative Agriculture

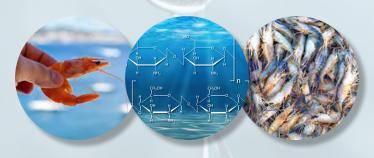
Regenerative agriculture is a set of practices that together promote and encourage a conservation mindset and rehabilitative approach to food and farming husbandry. Regenerative agriculture focuses principally on topsoil regeneration, increasing biodiversity in the soil biome by strengthening the health and vitality of farm soil, phyllosphere and the field in general, improving water cycling and usage, enhancing and revitalizing the agricultural ecosystem and supporting biosequestration.

Regenerative agriculture is not a specific practice. It combines a variety of sustainable agriculture techniques. These include but not limited to maximal recycling of farm waste and adding composted material from non-farm sources, restoration ecology, and holistic management. Large farms practicing or converting to regenerative methods like "no-till" and/or "reduced till" practices.

Within this regenerative framework, Impact SIX chitosan is a natural fit and an excellent additive to the farmer's arsenal. Chitosan as an input has many attractive features that support its use as a bridge to regenerative agricultural practices. Chitosan is non-toxic and eco-friendly. It is a natural material produced by some living organisms (chitin is far more common). Chitosan in agricultural products is derived from industrial sources of chitin be it shellfish or fungal. Either way, the chitosan from these sources are the same. Chitosan is biodegradeable in the soil. There are many microbes that can sense and digest chitosan as a food source. This is an important facet of carbon cycling not just from added Chitosan but nematode eggshells, insect and fungal carcasses are a chitin source. Chitosan promotes the presence of beneficial microbes in the soil. This feature greatly enhances the synergy between crop plants and soil microbes. The promotion of beneficials leads to greater synergy with plants and supports not only a healthy rhizosphere but contributes to building a healthy soil. Chitosan works synergistically with arbuscular mycorrhizal fungi (AMF). Aside from

the known benefits of AMF, these microbes are the source of glomalin, a substance deposited outside the AMF structure. Glomalin affords many benefits to AMF and the soil such binding sand and clay particles together with organic particles building the soil and its structure. The glomalin and soil contribute to water retention, provide air spaces and resist erosion. Glomalin also has a protective role to the AMF against microbial attack. Glomalin is also an important component to the soils carbon storage capacity. Chitosan being cationic can also help with building and stabilizing soils by forming linkages with soil and clay particles. Chitosan is also antagonistic to a wide range and class of phytopathogenic biotic stress agents (fungi, bacteria, viruses, nematodes) further contributing to healthy crop plants. From our own extensive experience with growers routinely employing our products, we have seen soils improve from a general composition standpoint to promoting more beneficials and inhibiting pathogens. In short, chitosan helps redressing the balance of the soil microbiome in favor of beneficials and symbionts.

Aside from what's going on in the soil, chitosan also has biostimulatory and eliciting functions all of which contribute to a healthier crop more capable of withstanding and surviving the seasonal biotic and abiotic stresses. Healthier plants lead to more productive crops!



#### Impact SIX as a Nematicide

Impact SIX chitosan exhibits many modes of action against nematodes. When chitosan gets in the soil it activates certain beneficial microbes (fungi and bacteria). Among the various strategies available to these microbes, some of these microbes produce enzymes that digest chitin (chitinases). The result is twofold: elevated levels of these chitinases in the soil and promotion of these beneficial species Chitinases are also released by many plants as part of their defense mechanism against various pathogens and plant parasitic nematodes. Taken together, these factors create an environment in and around the root zone that is detrimental to nematode establishment. Chitin is a component of nematode eggshells, pharynx and cuticle so these structures are targets for chitinases. The breakdown of the nematode eggshell chitin by chitinases can cause premature hatch, resulting in fewer viable juveniles. Ingested chitinases by females and juveniles can affect the pharynx and be lethal to embryonic nematodes, females laying defective eggs or moulting failure. The cuticle can be attacked by free chitinases and nematophagous (beneficial) microbes. Thus, the lifecycle can be interrupted (increased egg mortality) interrupted feeding with the result of having deceased and reduced active nematodes in the root zone. In addition, an elevated population of these beneficials minimize harm by space and resource competition, by providing higher nutrient and water uptake to the plant, or by modifying the root morphology, and/or rhizosphere interactions, that constitutes an advantage for plant-growth.

#### Impact SIX as a Fungicide

Impact SIX chitosan effects on phytopathogenic fungi appears to be multifaceted. Chitosan due to its cationic nature can bind to cell surfaces. This binding can restrict the free movement of materials in and out of the cells. Being cationic, chitosan can also bind to necessary and essential trace elements, interfering with the normal uptake of these nutrients and affecting the normal growth of fungi. Chitosan can also diffuse through the cell wall and interact with the plasma membrane. If this happens, chronic permeabilization (pore formation and severe disruption of plasma membrane components) occur. Such chronic damage causes cell leakage, the cell losing vital components, homeostatic balance and integrity and severe loss of membrane function. Such a situation is lethal. Should the chitosan gain entrance to the cell interior, there are many essential components that carry negative charges. Among these are proteins and nucleic acids (DNA and RNA). Binding with chitosan will drastically affect cell function affecting genetic expression mechanisms and protein biosynthesis. Chitosan is also known to increase oxidative stress in sensitive species leading to damage of essential cellular components.

#### Impact SIX as a Bacteriocide

Impact SIX chitosan action as a bacteriocide emulate those of the fungicide in several ways. Like fungi, bacteria are quite variable. Bacterial cell walls are of a different architecture and composition than fungi. There is no chitin in bacterial cell walls. Bacteria can be broadly divided into two groups based on cell wall composition. These types are known as Gram positive and Gram negative and is based on the peptidoglycan content of the cell walls. Basically speaking, Gram positive bacteria have a thick peptidoglycan (PG) layer than Gram negative. For both, negative charged components are embedded or coat this peptidoglycan layer. Chitosan as a polycation can bind to and envelope bacterial cells essentially starving them of essential nutrients. Chitosan can also chelate various essential nutrients and metals like zinc, copper, cobalt, manganese, nickel and cadmium. Chitosan permeability through cell walls interact with cell membranes and membrane components potentially resulting in catastrophic conditions of cell leakage, osmotic imbalance, oxidative stress, and interacting with cellular components responsible for genetic expression and protein biosynthesis. The degree of chitosan's cationic nature positively correlates with greater antimicrobial activity. Chitosan definitely shows stronger inhibitory effect at lower pHs, with inhibitory activity weakening with increasing pH. Of course, not all bacteria are chitosan sensitive. Many species are able to recognize chitin and chitosan in the soil and secrete a family of enzymes collectively known as chitinases. These hydrolytic enzymes are responsible for breaking down sources of chitin where they occur- an important component of carbon recycling.

#### Impact SIX as a Biostimulant

Biostimulants is a term used to describe substances or microorganisms that contribute to improving plant nutrition and growth. Biostimulants are either synthetic or natural materials. They are applied to soils, plants and seeds. Overall, biostimulants improve a plant's tolerance to abiotic stresses and also promote increases seed, grain yield and quality. Elicitors are another class of substances that positively act on plants. Elicitors can also act as biostimulants, and conversely many biostimulants have elicitor effects. Whether it is an elicitor or biostimulant, both promote positive plant responses.

Numerous biostimulant activities are attributed to Chitosan. Impact SIX chitosan increases photosynthetic activity, tolerance to drought, salinity, temperatures stress (elevated heat and chilling/ freezing), increased activity of antioxidant enzymes, defense genes (particularly related to fungi and bacterial infections), enhancing plant growth under abiotic stress, improving germination rates and efficiency, increasing seedling survival, and consequently, improving plant resistance to pathogen infection thereby improving plant growth and productivity.

Plants can recognize chitosan on their cell surfaces via a through pattern recognition receptors which recognize microbial compounds, such as bacterial flagella or fungal chitin. It is the particular pattern within each compound that triggers a response with the plant. In the case of chitosan, a number of varied responses have been described.

Foliar application of chitosan increases the levels of abscisic acid (ABA) and H<sub>2</sub>O<sub>2</sub>. Both of these substances are believed to be signals for stomatal closure (along with elevated Ca<sup>2+</sup> in the stomatal cells) controlling water loss via transpiration through the leaves.

Chitosan application promotes an increase of Ca<sup>2+</sup> concentrations in plant cells and induces callose deposition in plants. Plants produce callose to isolate stress impact in the tissue locally by depositing a physical barrier.

Chitosan can induce resistance to abiotic stresses, including salt, drought and temperature stress. Chitosan has been found to act as a stimulator of plant defense responses to both wounding and pathogen infections. Exposure to chitosan leads to production of reactive oxygen species (ROS) and pathogenesisrelated proteins (PRP's). ROS include Nitric Oxide (NO) and Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>). These compounds are produced in response to chitosan foliar application and act as signaling molecules leading to production of PRP's. These PRP's protect plant tissues against infection. It appears that different molecular weights of chitosan lead to different levels of PRP induction, suggesting that different types of chitosan molecules trigger different perception in plants. PRP's of note are chitinases and other fungal cell degrading enzymes. Phenylalanine ammonia lyase (PAL) is another. PAL is found in plants, algae, ferns, and microorganisms. It plays a key role in plant development and stress response and is involved in the synthesis of many secondary plant products, including Flavonoids, Lignins (helps prevent lodging), Plant Hormones, Alkaloids, Salicylic acid, Phenolics, and Phytoalexins. Phytoalexins are antimicrobial compounds that plants produce in response to infection or other stresses. They are part of a plant's defense system that helps control invading microorganisms. Phytoalexins are low-molecularweight, toxic substances that plants produce to protect themselves from fungi, bacteria, nematodes, and other organisms.

The chloroplast seems to be the primary organelle for chitosan action. Chitosan is also known to increase chlorophyll content which correlates to an enhanced growth or increased net photosynthesis rate. Chitosan sensing at the cell surface sends signal(s) to the chloroplast where several responses are generated.

Seed priming with chitosan has demonstrated numerous benefits to the seed and seedling. Soaking seeds with chitosan allows the chitosan to penetrate the seed coat enter the seed interior. Upon germination, the seed, and by extension seedling, are already primed with chitosan and benefit from the very start.

Chitosan is also influences flowering. Foliar applications have led to early budding, increased number of inflorescences and elevated pigments such as anthocyanins.



#### A note on the importance of pH

pH is a numerical scale describing acidity (or alkalinity) in aqueous solutions. pH is a term utilized by many and perhaps not understood what the term or values mean. pH is an indication of the concentration of hydrogen ions (H<sup>+</sup>) generally in an aqueous solution. The pH scale runs from 0 (most acidic) to 14 (most alkaline). The smaller the pH value the more acidic a solution is, so the pH scale is an inverse relationship with H<sup>+</sup> concentration. pH values are determined from a logarithmic scale where a pH change in one whole value is a 10 fold increase (or decrease) in H<sup>+</sup> concentration and vice versa. This relationship is expressed as follows:

$$pH = -log_{10}[H^+]$$

square brackets indicate concentration and the negative sign indicates the inverse relationship between pH and H<sup>+</sup> concentration So, a solution of pH 4 has 10 times more H<sup>+</sup> than a solution at pH 5. Conversely it can be stated that a solution of pH 5 has 10 times less H<sup>+</sup> than a solution at pH 4. Likewise, a pH of 2 has 10<sup>3</sup> (thousandfold) greater concentration of H<sup>+</sup> than a pH 5 solution. pH is an important consideration for agriculturalists with regards soil and spray tank mixtures. No need to expound on that.

From a chitosan perspective, pH is very important consideration to a spray tank mixture. Chitosan is a polycation. This means it carries multiple positive charges along the length of the polymer chains. The degree of this cationic nature is dependent on pH. Basically, the more acidic, the more cationic the chitosan becomes. It is the cationic nature of chitosan that is at the heart of its amazing variable functionality. Obviously, there has to be a balance between practicality, applicability and potential harm to target crop (very low pH). With chitosan, increasing pH (becoming more alkaline) the positive charges along the polymer chain gradually disappear. At around pH 6.2-6.3 a significant proportion of these positive charges disappear and chitosan solubility diminishes fairly rapidly. An important point to note is if the polycationic property of chitosan is deprived or reversed (for example by elevating pH), the corresponding antimicrobial (and so many other) capacities will be weakened or lost. Chitosan's effective cationic functionality occurs at pH below 5.0. This is an important consideration for the spray tank pH (chitosan solubility) and one of the main reasons Organisan Corporation recommends acidifying spray tank mixture pH to 5.0 or below (to ensure optimal chitosan functionality).

#### Brought to you by



# **The Pioneer in Agricultural Chitosan Technology**

Our proprietary manufacturing operations are located in Broussard, LA close to where our scientist lives. The "science" behind the formula was created, developed and is manufactured under the supervision of Dr. André Blanchard. Originally from south Louisiana, André spent most of his young life growing up in Inverness, Scotland (you'll be treated to both accents). He attended the University of the West of Scotland getting his bachelors in Applied Biology. André went on to gain his Ph.D. in Plant Molecular Biology at the University of Exeter in southwest England. From there, he returned home in 1992.

André brings a combined 25 years experience in academia and the private sector. André has worked with industrial scale recycling technologies and specialty chemicals manufacture. Within these industries, he has gained experience in directing product and process research and development. These efforts led in the technical development of a process (now a US Patent) for manufacturing a key raw material. André is also experienced in small business management, consulting, technology transfer, commercializing technologies, project management, process design and manufacturing strategies. He also initiated several collaborative projects with leading universities involving several external grant funded efforts from Federal agencies. André's association with chitosan over the past 18 years has involved researching and formulating new products, designing manufacturing processes, marketing and commercialization.

André is leveraging his experiences to leading future innovations of a variety of products, and constantly improving the manufacturing process.



Dr. André Blanchard, Ph.D., Plant Molecular Biology Vice President, Technology and Operations, Organisan Corporation Extensive Chitosan research and application for 30 years.

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